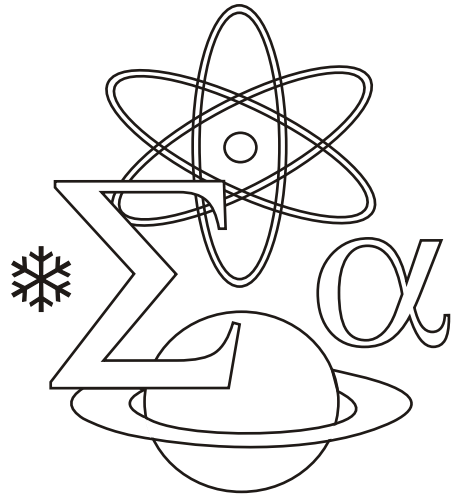
JNTS

JOURNAL OF NATURAL AND TECHNICAL SCIENCES



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Aims and Scope

This Journal is a multidisciplinary publication devoted to all field of Natural and Technical Sciences. The Editor of JNTS invites original contributions which should comprise previously unpublished results, data and interpretations. Types of contributions to be published are: (1) research papers; (2) shorts communications; (3) reviews; (4) discussions; (5) book reviews; (6) annonuncements.

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EARLY DETECTION AND SCREENING OF CERVICAL CANCER: MINISTRY OF HEALTH POLICY

Klodian RJEPAJ
Ministry of Health, Tirana, Albania

The Ministry of Health is committed on the continuous improvement of reproductive health status of citizens in Albania, in particular women, children and young people and providing to all the citizens an equal opportunity to meet their reproductive and sexual rights. To reach every individual, especially every woman, child and young people, Ministry of Health should undertake essential interventions on health and wellbeing in close collaboration with both national and international partners.

In the last 5 years, several interventions on improvement of sexual and reproductive health have been undertaken in our country. The legal basis for the components of sexual and reproductive health has been prepared. In addition, several national programs in partnership with civil society, national and international organizations have been implemented. Moreover, the IEC interventions at community and family level have been carried out to change the behaviours toward the reproductive sexual health, maternal and child health, IST, HIV and AIDS, reproductive tract cancers, etc.

With the support of the international agencies and in collaboration and partnership with civil society, public and health professional organizations, the health care staffs responsible for the reproductive health service have been trained on family planning, maternal and child health care, screening and prevention of IST, HIV and AIDS, reproductive tract cancers.

Even though the epidemiological data on cervical cancer indicates a lower incidence compared to incidence of breast cancer, there are two elements why cancer cervical should consider as a priority: i) the young age of onset of disease compared with other types of cancers and the high level of years life

lost (YLL) due to cervical cancer and, ii) the cost effectiveness of early detection / screening of cervical cancer is high. The scientific evidence on cervical cancer screening program considers it as the highest effectiveness program in reducing the mortality among the screening programs. The coverage level of cervical cancer screening is the only indicator of the screening programs included in the core indicators on the control of non communicable diseases (as it is defined by WHO).

In Albania, the Oncology Service at University Hospital 'Mother Teresa' reports about 100 new cases of cervical cancer annually and more than a third of them end up in death. More than half of cases are diagnosed in the advanced stages when treatment is more expensive and less effective.

Nowadays, it is already known that breast and cervical cancers are the main threats to the women health in our country. In Albania, the breast cancer is the most common cancer in women, with a steady increase of incidence, while the cervical cancer is the second common cancer in women of reproductive age (15-44 years old).

The early detection/screening of the breast and cervical cancer is considered as one of the priorities in Albanian Government Program 2013-2017, National Plan of Cancer Control and National Strategy of Reproductive Health.

However, some women have not yet the opportunity to be included in the cervical cancer prevention programs and therefore they are diagnosed in very late stages when the possibility of benefiting from treatment is low. So, the inclusion of the early detection/screening of cervical cancer in the primary health care service should be considered as a priority.

The services for the prevention and control of cancers of the reproductive tract, cervical cancer specifically has been included recently in package of primary health care services. Besides the approval of the clinic examination practice on the early detection of breast cancer in primary healthcare service (PHC), Institute of Public Health in collaboration with the National Centre of Quality Safety and Accreditation of Health Institutions has prepared the national guidelines and protocols on screening of cervical cancer in PHC.

The guidelines and protocols approved by MoH address four level of health care: i) community, ii) health centre or primary health care service, iii) regional hospital or second health care service and, iv) central hospital or tertiary health care level service.

The national guidelines and protocols define the tasks and required skills for the health care staff in the PHC. The health care staff in PHC will be oriented on four main areas of early detection /screening of cervical cancer:

- Informing, education, awareness of women on the importance of early detection of cervical cancer
- Collection and manipulation of specimen for cytology and HPV
- Coordination within the health system structure, including laboratory of cytology, biopsy and gynaecology service.
- Counselling and support for the diagnosed and treated women for cervical cancer.

The guidelines and protocols will increase the quality of services toward women through providing the same approach and standard for every women everywhere. They will increase the capacity of health care staff in PHC on the best practices and thus will enable the introduction of indicators on the performance of physicians on the early detection of cervical cancer. Furthermore, the guidelines and protocols will constitute a good foundation on the establishing of the early detection/screening program of cancer in our country.

The screening of cervical cancer should be performed at least once for every woman involved in target age group where the age group of 30-49 years old should be prioritized. Screening of cervical cancer is recommended at least once for every woman at age group target but it can also be extended to women younger than 30 years if a high risk of CIN2 + is present. In addition, testing for HPV, cytology and visual inspection with acetic acid (VIA) are recommended as screening tests.

In order that preventing cervical cancer program to be effective, all the women with a positive result in screening should be a subject who needs to be treated. The strategies "*screen and treat"* and "*screen, diagnose and treat*" are recommended.

A national program on cervical cancer screening is important in Albania. Ministry of Health is the leader on the process of the establishing of national program on of cervical cancer screening and the board nominated by Minister of Health will ensure the implementation of the program. National Centre of Quality Safety and Accreditation of Health Institutions is responsible for preparation of the standard; the continuous improvement of quality; and monitoring and evaluation indicators of the screening program. The closed

collaboration with partners in national and international level should be considered as an important element during the establishing of the national program.

The upcoming Decision of Counsel of Ministers on the package of national program of cervical cancer screening aims to ensure the providing of a free service on the early detection of precancerous conditions of cervical cancer and prevention of cervical cancer for women of age 30-50 years old at primary health care level and at secondary level of health care-public service (for women resulted positive at control at primary health care level). The screening program will expand in the national level within three years and it should guaranty the sustainability, high rate of coverage and quality of service.

The tasks are challenging and resources are limited and therefore difficult decisions need to be taken, but they are obligatory because they reflect the reality and are taken on the basis of the situation and the experiences of those working in the field of sexual and reproductive health in our country, as well as on contemporary evidence and international recommendations.

COMPREHENSIVE PREVENTION AND CONTROL OF CERVICAL CANCER

Artykova Nazira POOLATOVNA
WHO Representative in Albania
Gazmend BEJTJA
Public Health Officer, WHO Albania

ABSTRACT

Cervical cancer is one of the most common malignancies among women. According to WHO average 270,000 women die from cervical cancer annually. WHO and UNFPA globally and in Europe promote comprehensive prevention and control through the life course approach. Comprehensive systems for cervical cancer prevention and control have improved over the past 30 years, which has led to a significant decline in the morbidity and mortality of cervical cancer.

There are three stages in the life-course approach. Primary prevention includes counselling, vaccination health education of adolescent girls. Secondary prevention consists of comprehensive screening of women in 30-60 years age and treatment of pre-cancer conditions. Tertiary prevention means provision of care to all women above 60, as needed, treatment of invasive cancer through ablative surgery, radiotherapy, chemotherapy and palliative care. Only through screening and prevention nearly 530,000 new cases of cervical cancer could be prevented.

Cost effectiveness of comprehensive screening Programme for cervical cancer was approved and published in many countries developed and developing across the world.

Key words: cervical cancer; comprehensive life-course approach; screening

Reference: http://www.who.int/life-course/news/events/world-cancer-day-2017/en/

INTERNATIONAL SCIENTIFIC CONFERENCE ON CERVICAL CANCER PREVENTION, DIAGNOSIS AND TREATMENT

Manuela BELLO
UNFPA Albania

Excellency Minister of Health of Albania Mr. Ilir Beqaj
Excellency Minister of Health of Kosovo Mr. Ahmet Rrahmani
Excellency Deputy Prime Minister and Minister of Health of Macedonia Mr. Nikola Todorov
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President of the Albanian Academy of Sciences Prof. Muzafer Korkuti,
Representative of WHO in Albania, Dr. Nazira Artykova
Distinguished members of Honorary Committee
Members of the Scientific Committee
Members of the Organizing Committee
Professors from the European Health Institutions
Professors from the University Hospital Centre "Mother Tereza"
National Public Health Experts
Media Representatives

Ladies and Gentlemen,

I am most happy to join this distinguished forum aiming to help raise awareness and strengthen collaboration among key stakeholders on cervical cancer, a scourge affecting women across the globe.

Cervical cancer incidence in Albania is estimated 2.7 and mortality 1.7 per 100,000 (International Agency for Research on Cancers, 2012). Mortality rate from cervical cancer among women 15-45 years old comes second, surpassed only by breast cancer. Organisation, structures and capacities for early detection services remain weak; screening is not systematic and covers less than 10% of target population.

UNFPA has been supporting and will continue to do so in the future initiatives for building more organised and systematic health care services in the field of cervical cancer. I would like to highlight some of the key

initiatives supported by UNFPA over the last 5 years in strong collaboration with national and international actors:

Development of Policy analyses / documents with recommendations (road map) for the implementation of Breast and Cervical Cancer Prevention Programs in Albania.

Conduct of a national survey on health system gaps and capacities (assessing technical and human capacities on cervical cancer screening and diagnoses in all regions of Albania).

Development of a technical advisory document (memo for Minister of Health) highlighting priorities for immediate and longer term interventions in preventing and controlling cervical cancer in Albania.

'Declaration of Wisdom' symbolically signed by a large number of public figures, including Ministers, Deputy Ministers, Members of Parliament, health professionals and activists, mostly women.

Order of Minister of Health issued on 'Free services for patients affected by cancer'. It aimed to ease the access to upper levels of health care (including diagnoses and treatment) for all patients suspected to have cancer. It is expected to improve the utilisation of early detection services for cervical and breast cancer, etc.

Awareness campaign on cervical cancer and a national workshop organised by Ministry of Health (MoH), IPH and University Hospital (UH) where a technical document with 10 priorities was agreed upon (The Decalogue) in advancing interventions in the field of cervical cancer early detection and vaccination.

An accredited 4-days training course on cancer screening programme management focused on cervical and breast cancer screening management was organised by IPH in collaboration with Italian experts from Cancer Screening Center of Regione Piedmonte. 40 public health specialists from Tirana and all regions of the country were trained.

Cervical cancer screening included in the basic package of Primary Health Care Services.

MoH approved and endorsed newly developed guidelines and protocols for Primary Health Care provision of cervical cancer screening services in December 2016.

A training course which served to prepare the way for starting the piloting of the systematic cervical cancer screening programme was organized for professionals of women consulting rooms on cervical cancer screening,

A small scale pilot programme was supported in the regions of Tirana and Fieri; 2 accredited three days training courses were delivered and the skills of 92 primary health professionals in Tirana and Fieri were increased on issues such as informing women, taking/manipulating samples, referring positive cases and counselling. Three primary health centers were supported to provide

cervical cancer screening services for more than 1000 women in an coordinated effort of public health, primary health care professionals, hospital professionals and lab professionals. Experience on organized cervical cancer screening services gathered by IPH, University Hospital, and other health care institutions.

Objectives in our joint future efforts / actions:

Including all the regions into the cervical cancer screening programme;

Capacities of health care experts at all levels of system enhanced; I want to highlight here that based on the assessment of national capacity development needs in EECA countries, UNFPA EECARO established a cooperation with the International Federation of Cervical Pathology and Colposcopy (IFCPC) aimed at the knowledge sharing and institutional capacity building for cervical cancer prevention in EECA countries. As a result of this cooperation, the International Online Course for Colposcopy and Cervical Cancer Prevention will be supported during 2017 - 2018, aiming to strengthen national capacities in the EECA countries for cervical screening and clinical management of precancerous lesions in accordance to international standards. Two national experts have received the training of trainers in Lyon, France and 18 national experts representing all the country have been identified based on set criteria and have registered for this online course.

Quality insurance system in place and functional;

National cervical cancer screening program reaches its final target of 70, 000 women tested per year;

National program periodic report prepared;

Primary health centres, regional hospitals and public health directories systematically supervised and supported with training, advice and logistics aiming an effective national cervical cancer screening programme in Albania.

Dear colleagues,

Cervical cancer is the second most common cancer type among women in the world. Every year, there are more than 38,000 new cases and 18,000 deaths from cervical cancer registered in Eastern Europe and Central Asia (EECA) region. More than 80% of cervical cancer new cases and deaths are preventable, if national capacities are strengthened for well-organised cervical screening programmes and timely treatment of pre-cancerous lesions. Comparing to other screening programmes, the impact of cervical screening programmes is much higher, and it is evident, that in the countries with well-established cervical screening programmes the mortality and morbidity indicators are decreasing significantly. In Western European countries, the number of new cervical cancer cases and deaths is ten times lower than in EECA.

Ensuring healthy lives and promoting the well-being for all at all ages is essential to sustainable development. We, at the UN will continue to work closely with Ministry of Health and other stakeholders in achieving universal health coverage, including financial risk protection and access to quality essential health-care services paying more attention to the needs of disadvantaged and marginalized populations.

This important forum, with the participation of distinguished international and national experts in the field, emphasizes the power of coordination, partnerships, joint actions and active engagement of all key stakeholders and can surely catalyse effective change for women and our societies.

Thank you!

CHALLENGES AND PROGRESS IN THE FIELD OF CERVICAL CANCER SCREENING IN ALBANIA; LESSONS LEARNED AND PROPOSALS FOR ACTION

Alban YLLI
Institute of Public Health, Tirana, Albania

ABSTRACT

Cervical cancer is the second most frequent cancer among young women of reproductive age (15-44 years old) in Albania. There is an increased exposure to risk factors related to cervical cancer, although it is not reflected into an increased incidence or mortality, most probably because of better awareness about early detection. Age-standardised incidence is lower than other countries of Eastern Europe, but comparable and sometimes higher than Eastern Mediterranean Countries. Less than 100 cases are reported to be diagnosed each year. Mortality-to-Incidence ratio is estimated 0.4, much worse than the average of EU countries. There is difference in the quality of information between breast cancer and cervical cancer in Albania; for Breast cancer there is a centralized tertiary care in Tirana University Hospital Center, dealing with final diagnoses and treatment (despite the fact that still there are many cases treated abroad, in private hospitals or elsewhere in the country). For the cervical cancer there are at least two other university hospitals (gynaecological hospitals) involved in diagnoses and treatment) and only last year Institute of Public Health has started to coordinate the work for getting all the data from all hospitals. Similarly, breast cancer data received from family medicine can be used as a proxy for the prevalence of disease in population while the same data cannot be used for cervical cancer registered by family doctors. Regarding the mortality, data are unreliable since 2009. Similarly, GLOBOCAN cervical cancer incidence estimations for Albania has shown high variability during the last 8 years. Although cervical cancer is a much less frequent diseases in Albanian compared to breast cancer, its early detection programs, remain a priority because it is considered highly cost effective. After the development of the national country cancer program in 2011, some activities as been undertaken toward the building of a cervical cancer organized screening program in Albania. First policy analyses and preparation of first national report on cervical cancer control situation and recommendations was made possible during 2012 with assistance from European Association for Cervical cancer screening and supported by UNFPA. A national survey on health system gaps and capacities in the field of management of cervical lesions was carried out for the first time in 2013. Technical and human capacities on cervical cancer screening and diagnoses in all regions of the country were assessed. It was the first joint collaboration between

Institute of Public Health and University Hospitals. A detailed report with findings and recommendations was prepared after a national workshop. 2 specialists from Center for Cancer Screening of Regione Piedmonte (a collaborative WHO center) were invited in the workshop as well. A 6-page technical advisory document were prepared and sent by IPH to Ministry of Health. It highlighted priorities for immediate and longer term interventions (organisational, capacity building, technology and running costs). 'Declaration of Wisdom' was symbolically signed by a large number of public persons, including Ministers, Deputy Ministers, members of parliament, health professionals and activists, mostly women. It is a document which states goals of an alliance against cervical cancer to reduce deaths from that cancer within the next 5 years, and to give to the women of coming generation's better life free of cervical cancer. In January 2014, a technical document with 10 priorities was agreed upon by many Albanian and international professionals. It contained a common vision for necessary activities and interventions in the field of early detection and vaccination. The need for organised screening and the role of primary health care were underlined among other issues. For the first time an accredited 4-day training course on cancer screening programme management focused on cervical cancer screening, breast screening and colorectal screening management was organised by IPH in collaboration with Italian experts. 40 public health specialists from Tirana and all regions of the country were trained. In 2015 IPH and the National centre for accreditation and quality has signed a agreement and set up an working group to prepare primary health care guidelines and protocols for cervical cancer screening. For the first time a series of training courses (3 in total in Tirana and Fieri) for professionals of primary health care on cervical cancer screening was carried out during 2016. During that year a TAIEX-supported workshop with EU experts discussed technical aspects based on the EU most recent Guidelines. Despite the declared priority into policy documents, there is no yet an organized program of cervical cancer screening in Albania. Only opportunistic tests are provided. Around 5000-10 000 tests are done each year. Reading of cytology is centralised in Tirana. Colposcopy is carried out only in Tirana clinics also. Based on newly developed country protocols and on the most recent EU and WHO guidelines Ministry of health as agreed on a plan to start large scale piloting of organized screening program based on primary health care services which will be responsible on informing the targeted women, taking the cervical samples and coaching the women in higher levels of health system. Directories of Public health will support the logistics of the program. For its efficacy, practicality in quality monitoring and long term cost-effectiveness, HPV test is planned to be used as a primary screening test. Tirana and Fieri regions will be included in the first year (2017) pilot program with the objective to reach 30 000 women per year. From 2018 scaling up of the program to other regions will start with the objective to reach 70 000 women per year by 2020. During that period, quality insurance system and monitoring will become functional and capacities in all levels of the system will be enhanced.

Keywords: CVC, treatment, action, Albania, Europe

1. INTRODUCTION

Cervical cancer can affect all adult women until very old age, but it is most common in the age group 30-50 years. It is rare under 25 years old.

Among female, cervical cancer is one of the ten most frequent cancers. European Union Mediterranean countries have an average incidence of 7.1 per 100,000 women with a mortality to incidence ratio of 0,25. Among the non-EU Mediterranean countries the incidence rates exceed 10/100000 women in the Balkan countries while they are very low in the remaining regions. The mortality to incidence ratio is higher than 0,5 in Libya, Morocco, Algeria, Egypt, Jordan etc. Albania's incidence and mortality are lower than most Balkan Countries but higher than most Eastern Mediterranean countries (Giordano *et al.*, 2015). While cervical cancer incidence in Albania is comparable with the average of EU Mediterranean countries, mortality is much higher.

Albania is a European Union candidate country characterised by transitional economy. Only about half of its 3.6 million people are covered by health insurance, although primary care is highly accessible to the whole population through approximately 400 publicly financed healthcare units. Cancer diagnosis and treatment since 2014 are provided free of charge, but most services are concentrated in the only oncologic centre in the country, in the capital city of Tirana (Anttila *et al*., 2013). More than half of all breast and cervical cancers are diagnosed at an advanced stage (III or IV), due to insufficient access to diagnostic and cancer management services for a large proportion of the population.

Organized cervical cancer screening programme does not exist in Albania. Only 5,000 – 10,000 Pap tests are taken and read annually in an opportunistic way, not organized and mainly private. Meanwhile the biopsy and treatment of precancerous lesions (using the loop electrosurgical excision procedure – LEEP) are limited to a few centres in the city of Tirana. The maternal health services network is well developed: it could provide an organizational backbone for these screening services, provided that screening technologies suitable for the skill-sets of maternal health service providers were selected.

There is a large experience from industrialized countries and developing countries as well about screening programs organisation and screening test validity and practicality (Coleman *et al*., 2008). European Union has updated its guidelines on Quality Assurance on development cervical cancer screening tests in 2015 (von Carsa *et al*., 2015).

The guidelines underline that "There is clear scientific evidence that a screening based on validated tests for the DNA of oncogenic HPV as primary test and applying an appropriate protocol is more effective than screening based on cytology in preventing invasive cancers of the uterine cervix. In

addition, it entails a limited - if any - increase of the undesired effects both in terms of unneeded referral to diagnostic work-up and in terms of over-diagnosis and consequent overtreatment of spontaneously regressive lesions." (IARC 2012).

World Health Organisation (WHO) has developed in 2014 an updated version of guidelines for cervical cancer control on primary health care settings.

Some crucial elements of EU and WHO guidelines are the followings: i) the need for triage for HPV+ women (cytology, if positive colposcopy), ii) at least 5 year interval after negative test: the 5-year cumulative risk of high-grade CIN after a negative HPV test is lower than the 3-year risk after a normal cytology, iii) HPV-based screening should not start before 30-35 years, iv) only tests for the DNA of oncogenic HPV, validated according to the European policies and, v)no double testing with cytology and HPV.

Some of these principals are already included into the Albanian technical documents and guidelines described in this report

2. METHODS

In this paper, analyses of the burden of cervical cancer in public health and society as well as its trends in time and geographical distribution in Albanian regions are provided. This analysis is based on various data from University Hospital Registry, General Practitioner's registries and estimations from GLOBOCAN. Indicators are age standardized when comparisons are made among countries and rates are given per 100 000 inhabitants.

To calculate the costs of the problem we used mortality and incidence data, average age of diagnoses, Albania's Gross National Product per capita and some data of treatment costs.

To describe the progress and gaps in the health system related to cervical cancer early detection, reports of recent (last 5 years) interventions and various technical unpublished documents have been used. Interventions reviewed have included health system analyses, capacity building, networks, awareness, and Primary Health Care (PHC) based interventions. Lessons learned, gaps and strengths were identified, while evidence based components to be taken into consideration for further progress was highlighted.

To estimate the awareness in general population, Google Trends data from Albania were used as a proxy of public interest about the term 'kanceri i qafës së mitrës' and 'kanceri i gjirit'.

3. RESULTS

Trends and distribution of the problem

As it is presented in the figure 1, Albania has a relatively high mortality from cervical cancer compared to its actual incidence which indicates a poor control of the disease including late detection and ineffective treatment. Cervical cancer mortality rate in Albania is 55% higher than the average of mortality rate in EU, while the incidences are comparable.

	Incidence	Mortality
Serbia	20.9	9.2
Morocco	14.1	8.4
Montenegro	13.3	6
Croatia	11.8	4.2
Algeria	10.4	6.1
Bosnia and Herzegovina	9.1	3.7
Albania	7.1	2.8
Mediterranean EU Countries*	7.1	1.8
Tunisia	6.3	3
Turkey	4.2	1.6
Lebanon	3.8	1.8
Jordan	3.6	1.9
Syria	2	0.8
Egypt	1.6	1

Fig. 1. Cervical cancer in Albania and Mediterranean Countries. Age-standardized incidence and mortality (/100 000 inhabitants**).**

Although cervical cancer burden in health remains quite low compared to breast cancer, it is only second to breast cancer among the women of reproductive age (IARC 2012). Figure 2 depicts this fact by means of age-standardised incidence of various cancers among women 15-44 years old.

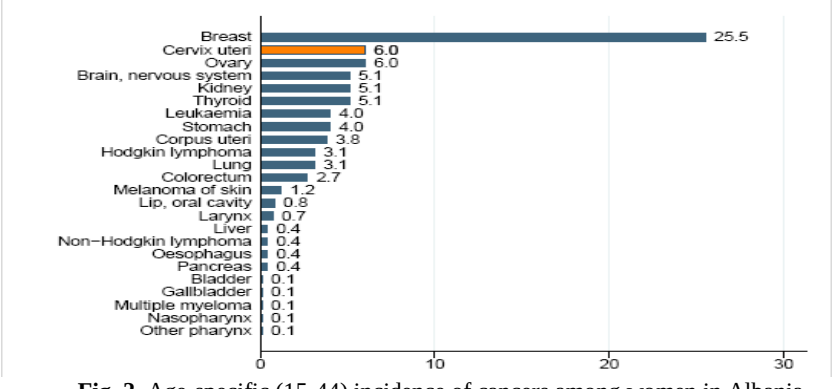

Fig. 2. Age-specific (15-44) incidence of cancers among women in Albania.

While in most EU countries mortality and incidence of cervical cancer are on decrease, in Albania the incidence trend remains stable with a slight tendency for increase. The data from University Hospital Registry are in the figure 3 depicted.

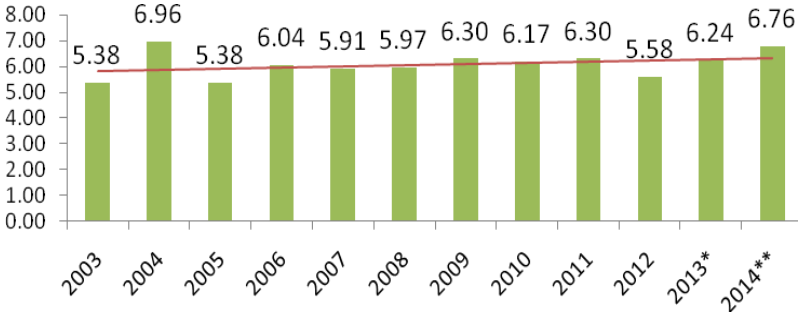

Fig. 3. Time trends of incidence rates for the period 2003 -2014. Hospital's based registry. Incidence rate /100000

Not all the regions of Albania have the same rate of the diseases; when data from general practitioner's registries were analyzed it showed a concentration of cases in most urbanized areas of the country, Tirana, Durrës and Korçë, followed by Shkodra, Fier and Elbasan. As there were no registered cases in Saranda (data not shown) for all 5 years under study it can be hypothesized that potential cases from these areas close to the border might be treated abroad Figure 4.

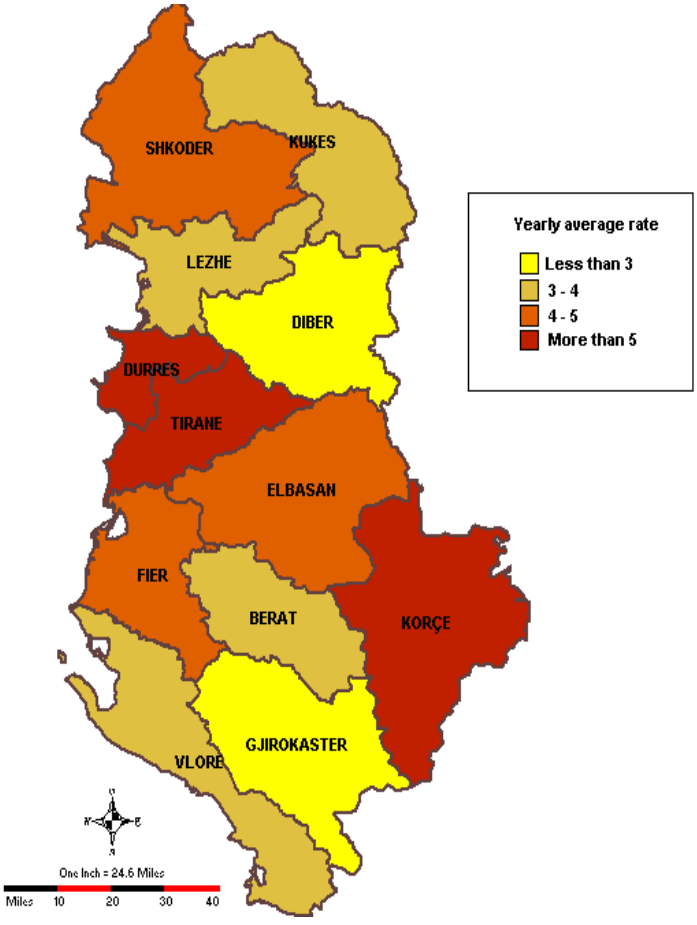

Fig. 4. Regional distribution of average yearly rate for the period 2010-2014. Data from GP registries. (/100 000)

Cost of cervical cancer on Albanian society

Cervical cancer related deaths in Albania cause a loss of approximately 1150 life years per year. (Average life expectancy among women 78 years (6), average age of death 50 years resulting in around 28 years lost per every woman. An average of 40 deaths per year would produce around 1150 Life years lost in total in Albania each year)

The burden is higher when focus is on disability adjusted lost years (DALY), because in this case we measure not only the deaths but the disease and its debilitating potential also into quality of life of persons. We estimate more than 4000 disability adjusted lost years from this disease every year in the country (around 100 new cases per year with an average age of diagnoses

at around 40 years old causing approximately 39 healthy life years lost for each sick women and around 4000 disability adjusted lost years or DALY).

Burden of disease is spread from health to economy in many ways and the most direct one is related to loss of economic productivity because of disease and death. From 600 to 2600 productive life years are calculated to be completely or partially lost every year from the cervical cancer (death will cause 15 productive life years lost for each women dead at 50 years of age while women could have been otherwise productive till 65 years of age. On the other hand, disease starting in average at 40 years old for more than 100 new cases in Albania would result in 25 years lost for each omen, or around 2600 productive years lost in total every year. This loss of productivity would result into a significant loss for Albanian economy calculated from 3000000 USD to 13000000 USD (based on the GDP per capita of the country calculated approximately 5000 USD). Other important costs for society are related to health care for the diseased, costs into the family and parenting with long term negative outcomes in education of children etc. These costs can be estimated to be another several millions of USD.

Screening interventions are calculated to be very cost effective or 'Best Buy' by WHO (2011) and in Albania are calculated to cost no more than 0.5 USD per capita in the most costly option, which would have been less than 1 500 000 USD per year for all the country. To be mentioned that in these costs are included all costs of the health system.

Policies and intervention during last 5 years

A number of health system based analyses, capacity building, awareness activities, policy development and guidelines preparation have been carried out during the recent years in Albania; in the 2010 an international workshop on cancer screening was organized with support from some of the best experts in that field in Europe. In 2011 National Cancer Control Plan was approved with an order of Minister of Health. During the same year Mediterranean Network, lead by Italian Ministry of Health and Turin Center for Cancer Screening started to provide support with workshops and comparative analyses among participant Mediterranean countries. In 2012 it was carried out first health system analyses on breast and cervical cancer screening opportunities with support from UNFPA and European Association of Cervical Cancer Screening. First national hospital-based study of capacities and gaps for cervical screening was developed and carried out by Albanian experts from Ministry of Health, University Hospital Centre (UHC) and Public Health Institute (IPH). A team of Gynecology, cytology and public health professionals prepared a report after visiting all regional hospitals and interviewing a large sample of health professionals.

Based on the experience and findings gathered from the system analyses, IPH in 2013 sent first technical document with specific recommendations on cervical cancer screening to Ministry of Health (MoH).

Few main points from December 2013 recommendations are listed below: i) there were underlined problems with efficacy, high risks and service ethics of existing opportunistic (wild) screening activities, ii) need for quality control and information system, iii) new role proposed for primary health care, women centers (integration with CBE at PHC level), iv) recommendations for centralization of test reading and balanced decentralization of colposcopy, v) recommendations to start organized screening gradually, vi) proposals for specific capacity building at various levels of the system, vii) serious consideration of HPV test as a primary screening test and, viii) advice to consider combining cervical cancer screening with new check up program.

During 2014 an important advocacy activity was organized with Declaration of Wisdom being signed by a number of politicians of all branches, including ministers, deputy ministers, members of parliament and other personalities of public life. The document underlined the need to do more to prevent cervical cancer and protect women's life from this disease.

Another national workshop focusing on screening on cervical cancer program in Albania was organized in 2016 by Ministry of Health with support from EU (TAIEX).

During the 2015-2016 period, an inter-institutional working group was set up and under the leadership of IPH and Center for Quality on Heath Care developed the guidelines of cervical cancer screening at Primary Health Care (PHC) level. The guidelines are approved by an order of Minister of Health in December 2016.

Capacity building activities in public health cancer control have started in 2014 with one 4 days accredited training course on screening programs management for public health professionals working at Directories of Public Health (DPH). In 2015 and 2016 three accredited (3 days) training courses focusing on cervical cancer screening practice at PHC and based on the newly developed guidelines and protocols, were provided for more than 120 PHC doctors and nurses in Tirana and Fieri regions. During 2016, following up the training courses some organized screening activities in three selected Health centers were carried out in Farka and Libofsha communities.

Awareness

From the figure 5 and 6 it seems that while the interest of the public about breast cancer (expressed by means of internet searches) has increased, the interest about cervical cancer doesn't show the same trend.

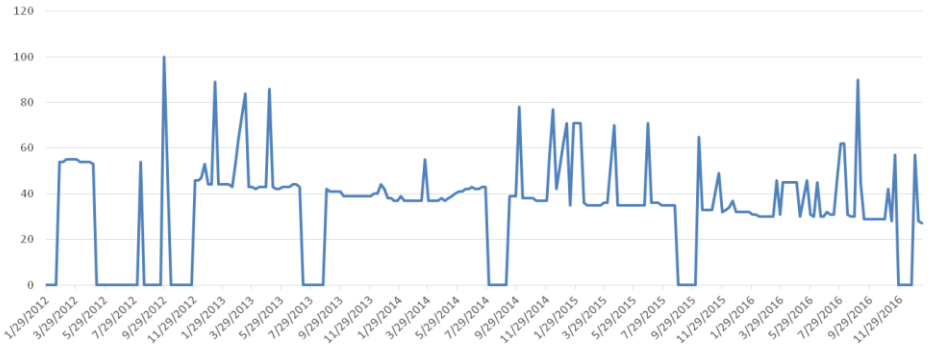

Fig.5. Weekly volume searches about 'kanceri i qafës së mitrës' in Google engine from 2012 to 2016.

Fig. 6. Yearly volume searches about 'kanceri i gjirit in Google engine during the period 2009-2015.

From the reports and analyses reviewed in this work, there can be summarized the following main challenges to the Albanian cervical screening program: i) opportunistic tests bring risks and financial costs for women, ii) low coverage and most probably the same group of women is tested every year, iii) no dedicated information system, iv) poor quality assessment, v) few qualified personnel at PHC and, vi) capacities on cytology and colposcopy very limited and centralized in Tirana.

4. DISCUSSION AND RECOMMENDATIONS

There are a number of lessons learned during the implementation of the pilot organized (quasi) limited programs in Albania.

PHC clinics can be the bases of the organized screening program, in urban areas and most rural areas. In rural areas gynecologic beds and midwifes are key for assuring the administration of samples at the health center settings.

Women clinics are the best settings for the primary testing activities in urban areas. Procurement of kits for tests at local level resulted impossible and is advisable that cconsumables or kits to be procured centrally (MoH) and distributed to health centers through Directories of Public Health.

A health center can easily invite and test hundreds of women per year. Frequency of women coming for testing and advice increased sharply at the beginning followed by stagnation. Nurses needed support for invitations, especially in urban areas.

HPV testing proved to be very practical and have been handled easily by health centers. Self-taking sampling is the easiest and most preferable approach by women and health doctors alike.

DPHs can play an important role in monitoring the process at every region (as well as supporting with logistics for transportation of samples).

Communication ethics and skills at any level of the system can affect the trust, the utilization and finally coverage. Quality of services varies greatly among hospitals and policlinics. The best way would be to send initially all positive women to university hospitals for cytology and eventual colposcopy. After some training or quality standardization, regional hospitals can take over the colposcopy examination.

Traditional information system could be a significant burden for health personnel. For large scale program, it should be substituted by a digitalized system. A small initial investment in technology can avoid the overburden on the health centres, hospitals and public health institute to manage the data (Ponti *et al*., 2017).

In rural areas women were contacted easier than in urban areas and they responded better well. Invitation process needs more support and more resources, especially, in urban areas.

Training and supervision of personnel will continue to be a challenge. It needs dedication of recourses.

In the way forward, setting up a large scale organized program in Albania would require support for four main components: i) sustainability (regulatory bases, MoH leadership); ii) safety and ethics (standards, pathways and responsibilities); iii) gradually (starting with large scale pilot in some regions) and, iv) efficacy (board, management, indicators of performance).

During this process the following main targets within the given timeline are recommended: i) 2017-2019: large scale organized pilot in selected regions (Tirana, Fieri, Dibra) followed by 2019-2020: scale up to national program and, ii) 2017-2018: 30 000 – 40 000 women and then during 2020-2021: over 70 000. Based on INSTAT data where women's population 30-60

years old is 611 208. Invitations to be sent to a estimated 122 242 women population in 5 years frequency.

Main stakeholders which will have a role in the new system and which will assure sustainability and quality of the program are Ministry of Health, University Hospitals and Institute of Public health, National Centre for Health Service Quality, regional hospitals and women centers, health centers and women clinics

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THE GERMAN HEALTH CARE SYSTEM AND ITS SCREENING CONCEPT FOR CERVICAL CANCER COMPARED TO OTHER EU COUNTRIES

Hans IKENBERG
Cytomol, MVZ for Cytology and Laboratory Diagnostics, Frankfurt, Germany

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Germany has obligatory insurances for the whole population. Basically, (more than 100) public health care funds are accessible for all. They cover over 90% of the population. Beside them full private insurances can only be taken by people above a certain income (≈6.000 € /month). Private insurances to upgrade the services of the public health care funds are available as well and used by more than 50% of their members.

Free screening for cervical cancer with annual conventional cytology (CC) starting at 20 years with no upper age limit has been introduced in Germany in 1971. Smears are taken by gynaecologists and analyzed by cytologists who still are to ≈ 70% gynaecologists in still a high number of mostly small labs (≈>500). The formally opportunistic system is now rather strictly quality and data controlled. The participation rate with ≈80% on a three year-base is on the same level as in countries with formally organized screening programs. In September 2016 the main regulatory institution (G-BA) decided a new screening approach which will be probably implemented within 2-3 years. A formally organized screening will rely on CC from 20 to 35 years. In women over that age a contesting with HPV and CC every three years will come. This decision overthrew an earlier decision which would have introduced HPV primary testing every 5 years or annual CC according to the women's intention. This reversal was the result of an often aggressive campaign of the professional societies of gynecologists, pathologists and cytologists against HPV primary diagnostics. A further consequence of that debate is the extreme delay in the publication of a new S3 guideline on cervical cancer screening recommending also primary HPV testing.

Regarding the EU roughly there exist two different medical systems. More privately organized (but more or less strictly state controlled and even driven) as in Germany, France, Italy or Spain. Public health care dominates in the

more Northern countries like in Scandinavia, the UK and the Netherlands. Here screening is formally organized with invitation of the women, later start (mostly 25 years), stop at 60-65 years, longer intervals (3-5 years), the smears are often taken by nurses and the labs are centralized. Following an EU guideline advocating primary HPV screening, more and more of these countries switch to HPV. However, up to now the change is often only in pilots or regional.

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HPV DIAGNOSTICS IN ROUTINE

Hans IKENBERG

Cytomol, MVZ for Cytology and Laboratory Diagnostics, Frankfurt, Germany

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Since more than a decade testing for High-Risk-(HR)-HPV-DNA has become a relevant element in the prevention of cervical cancer. Within the last years large studies of high methodic quality have further underlined the importance of HPV diagnostics. They demonstrated that for the detection of high-grade cervical preneoplastic lesions (> CIN2) HR-HPV testing is more sensitive but less specific than cytology and that HPV testing is less variable than cytology. Of capital importance is that women with a negative HR-HPV basic test have a much lower risk of developing a lesion > CIN3 than those with a normal cytology report.

There are three main fields where HPV diagnostics is already in routine use: 1) triage of cytological borderline and low grade abnormalities 2) follow-up of patients after therapy of CIN as a test of cure 3) as an adjunct to conventional and thin layer cytology in women above 30 years. All these indications are recommended by US and European guidelines. Meanwhile in some countries even the substitution of cytology as primary screening instrument by HPV testing is on the way.

HPV prevalence and elimination rate are high, especially in young women. Only HPV persistence over a certain level is of clinical importance. To achieve high sensitivity and specificity for CIN2+ standardized methods of HPV detection are of utmost importance. The balance between analytical (low) and clinical (high) sensitivity is crucial for the specificity of a routine HPV test especially in a screening approach. That is the reason why for a long time the HC2 test (Qiagen) was the only assay qualified for HPV routine diagnostics.

New tests for routine HPV testing must be validated according to a guideline defined by leading experts on the field. Meanwhile several systems have passed this hurdle. Three of them (a signal amplification test [Cervista, Hologic], a RT PCR [cobas, Roche Diagnostics] and a RNA test [Aptima, Hologic]) have also received a FDA approval. Currently the broadest data

base is available for the cobas test (Roche), the technique analyzed in the to date largest HPV study (47.000 participants).

HPV 16 and 18 implicate an up to five times higher risk for the development of a CIN 2+ as the other HPV HR types. It is therefore appropriate to test for these HPV types independently in parallel or after a positive HPV high-risk test. At present a more detailed HPV typing is not useful in routine. HPV RNA testing is an interesting option with probably higher specificity. Its clinical value still needs to be confirmed in long-term studies.

Even in the case of HPV 16/18 positivity the large majority of lesions will not progress to cancer. Therefore progression markers are valuable tools. Up to now the best validated test is the combined immunocytochemical detection of p16 and Ki-67 in the same cell (CinTec PLUS, [Roche MTM]).

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GLOBAL OVERVIEW OF THE VALUE OF HPV PRIMARY SCREENING

Martin KRESTANPOL
Freelance Consultant, Prague, The Czech Republic

ABSTRACT

Cervical carcinoma is the only cancer with known single cause – high-risk genotypes of human papilloma virus (HPV) (Walboomers *et al*., 1999). Most high-risk HPV infections occur without any symptoms, go away within 1 to 2 years, and do not cause cancer. Some HPV infections, however, can persist for many years. Persistent infections with high-risk HPV types can lead to cell changes that, if untreated, may progress to cancer (Schiffman *et al*., 2007). **Vaccination** can prevent infection by HPV vaccine types but not eliminate it once it occurs. Standard way to prevent cervical cancer is by testing with the Pap test (Papanicolaou test or Pap smear) to find and treat cervical cancer precursors — cervical intraepithelial neoplasia grade 2 (CIN2) and principally grade 3 (CIN3) - before they can turn into invasive cancer. Regardless of its lower sensitivity it is still one of the most effective screening tests and cytological screening has reduced the incidence and mortality of invasive cervical cancer especially in countries with high quality organised screening programs organised screening (Bray *et al*., 2005).
Keywords: HPV DNA test, HC2, QIAsure, MEthylation Test

1. INTRODUCTION

HPV DNA testing in primary screening – clinical validation

Recently, evidence from randomised trials supports integration of HPV testing technology into screening programs (IARC, 2005;Schiffman *et al*., 2007; Franceschi *et al*., 2009). The HPV based screening compared to standard of cytology is more effective in reducing the cervical cancer incidence after a negative screening test result and allows extending screening intervals (Ronco *et al*., 2014; Arbyn *et al*., 2012; Poljak *et al*., 2012). G. Ronco compared four European randomised trials comparing HPV-based with cytology based cervical cancer screening. Swedescreen (Nauder *et al*., 2007), POBASCAM (Bulkman *et al*., 2007; Rijkaart *et al*., 2012), ARTISTIC (Kitchner *et al*., 2009) and NTCC (Ronco *et al*., 2010) with more than 170 000 women resulted in 60-70% reduction in invasive cancer incidence, compared to cytology based screening. It also demonstrated that the

cumulative incidence of cervical cancer 5 years after a negative HPV test was lower than the incidence 3 years after a normal cytology result (Ronco *et al*., 2014).

A milestone in HPV-based cervical cancer prevention screening came in 2009. An international team of experts defined the criteria for assay validation in primary screening based on reproducibility and relative sensitivity and specificity compared to the *digene* HC2 HPV DNA or GP5+/6+ PCR–enzyme immunoassay (Meijer *et al*., 2009). The *digene* HC2 HPV DNA (HC2) and the GP5+/6+ PCR–enzyme immunoassay (EIA) are considered as fully clinically and epidemiologically validated (12). HC2 (QIAGEN, Gaithersburg, MD, USA) detects all 13 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) using the signal amplification method with full genome probe hybridisation (14). The GP5+/6+ PCR-EIA uses a single pair of consensus primers targeting a conservative DNA sequence of 140 bp of the L1 gene of HPV. Detection is carried out by hybridization with type-specific probes with the by EIA-based detection. It identifies 14 HPV types (the same types targeted by HC2, plus HPV 66) (Jacobs *et al*., 1997).

Recently, more than 125 HPV assays have been introduced to market. However, only few of them have been reported with clear evidence of their clinical utility (Arbyn et al., 2012; Poljak et al., 2012; Arbyn *et al*., 2015). Arbyn and colleagues (2015) verified which tests fulfilled these criteria and published the High Risk HPV Assays that fully matched the criteria set by Meijer at al. and that can be recommended in HPV- based cervical cancer screening programs: HC2, GP5+/6+, PCR-EIA, Abbott RT hrHPV test, cobas 4800 HPV test, PapilloCheck HPV-Screening test, BD Onclarity HPV assay and the HPV-Risk assay (Arbyn *et al*., 2015).

In low setting countries, a proof of the value of the HPV testing has been documented for population in rural India that did not benefit from routine high quality screening. In a cluster-randomised controlled trial that measured the effect of a single round of screening by testing for human papillomavirus (HPV), cytologic testing, or visual inspection of the cervix with acetic acid (VIA) on the incidence of cervical cancer and the associated rates of death, one single round of HPV testing, using the HC2, Gaithersburg, MD, USA, reduced the incidence of advanced cervical cancer and cervical cancer deaths and a single digene HPV Test within an 8-year period saved the lives of about 50% of women at risk of developing cervical cancer (Sankaranarayanan *et al*., 2009).

The *digene* HC2 HPV DNA Test

The HPV test should be clinically validated to predict the risk of cervical cancer and it precursors and thus to identify women in risk of development of

the cervical cancer. The far most thoroughly clinically validated HPV assay is Hybrid Capture 2 (HC2; Qiagen, Gaithersburg, MD, USA). HC2 detects all 13 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and 5 low-risk types (6/11/42/43/44). QIAGEN is one of the world's leading producers of molecular diagnostic solutions and is committed to reduce the morbidity and mortality associated with cervical cancer and to make the HPV tests affordable. The range of QIAGEN solutions allows all national public health programmes and private laboratories to implement testing despite differences in epidemiology, infrastructure, and service provision between countries. The partnerships with healthcare professionals, governments, scientists, and screening organisations, combined with advanced technologies, allow offering new hope and goal of eliminating cervical cancer.

As already mentioned the challenge with HPV testing for cervical cancer is to identify, consistently and reproducibly, clinically relevant high-risk HPV infections (clinical sensitivity), while minimizing reports of clinically irrelevant infections (specificity). HC2 technology has proven high sensitivity for detecting disease, while minimizing false positives (Arbyn *et al*., 2012). HC2 chemistry is designed to be robust and reproducible, and has been independently validated in numerous sample types and across multiple patient cohorts. Since its launch in 1999, the *digene* HC2HPV DNA Test has consistently demonstrated high sensitivity for CIN 2+ with a low rate of false positives and excellent reproducibility in various settings and in various indications. As such, the *digene* HC2 HPV DNA Test remains the global benchmark standard for HPV testing for cervical cancer diagnosis and screening.

An HPV test needs to be robust, use a clinically relevant threshold and offer proven reproducibility. In other words, the test should remain unaffected by the inherent variability of biological samples, avoid issues that can undermine target amplification techniques and detect the viral genome despite gene deletions. The *digene* HC2 HPV DNA Test remains the gold standard method for HPV DNA detection and is the most clinically validated HPV test in the world, supported by more than 500 high-quality peer-reviewed publications that enrolled more than one million women, including many independent randomised controlled trials with long-term follow-up data. These trials show that the clinical sensitivity of the *digene* HC2 HPV DNA Test for CIN 2+ is substantially higher than that of a Pap smear (19-21). Furthermore, women who test negative with the *digene* HC2 HPV DNA Test are highly unlikely to develop cervical lesions or cancer for the next six years. The HC2 assay is the commercially available assay supported by independent long-term data demonstrating a safe extension to the screening interval compared to Pap testing (Katki *et al*., 2011).

QIAGEN's *digene* HC2 HPV DNA Test delivers reliable HPV DNA detection by employing Hybrid Capture technology. The *digene* HC2 HPV DNA Test is highly tolerant to common inhibitors, including blood, that are often found in cervical samples. Adding specimens that are in digene Specimen Transport Medium (STM), Hologic PreservCyt® Solution, and BD SurePath® medium into the base solution disrupts HPV and releases target DNA. The target DNA combines with specific RNA probes, creating RNA: DNA hybrids. The RNA: DNA hybrids are captured onto a solid phase coated with universal capture antibodies specific for the target hybrids. The captured RNA: DNA hybrids are detected with multiple antibodies conjugated to alkaline phosphatase to allow signal amplification. The chemiluminescent reaction reveals the presence of HPV DNA.

HC2 can be automated to deliver high-volume screening efficiently and effectively. This facilitates the centralisation of HPV and cytology testing that is likely to be required in future HPV based cervical screening programmes.

The *digene* HC2 HPV DNA Test offers several technical advantages over other HPV assays. In particular, HC2 technology does not rely on sensitive enzymatic reactions and, therefore, is more tolerant to problems (such as contamination during sample processing) than tests based on target amplification technology. This is one reason why the infrastructure and training requirements to deliver HC2 testing are minimal compared to other technologies. In addition, the *digene* HC2 HPV DNA Test is less affected than other assays by the inherent differences and variability often found in cervical samples. As the disease progresses from early HPV infection through to persistent infection to CIN 2/3 to cancer, regions of HPV (including L1) can become deleted or mutate as viral DNA integrates with the host genome. Assays that target only this region may miss, for example, integrated HPV in which L1 genes are deleted (Morris *et al*., 2005; Hesselink *et al*., 2006). The ability to capture the entire genome helps make the *digene* HC2 HPV DNA Test to be sensitive HPV test for histologically confirmed CIN 3+ by detecting advanced infections characterised by the deletion of specific viral genome regions. HC2 is thus the test offering this safety feature to maintain sensitivity through all stages of HPV infection.

Studies assessing the *digene* HC2 HPV DNA Test provide much of the evidence base that supports implementation of HPV DNA testing in cervical cancer diagnoses. Several European countries (including The Netherlands, United Kingdom, and Italy) used this evidence to calculate the clinical efficacy and cost effectiveness of primary HPV testing in their screening programmes (Acceta *et al*.,2010; Rijkaart *et al*., 2012; Ronco *et al*., 2012). Based on these calculations, HPV testing followed by cytology as a triage test emerged as the most desirable screening algorithm providing the optimal combination of sensitivity and specificity. This approach offers sensitivity to

detect disease earlier than primary cytology test algorithms. High specificity of cytology triage allows to reduce unnecessary treatment referrals and to safely prolong the screening interval using HC2 (Acceta *et al*.,2010; Rijkaart et al., 2012; Ronco et al., 2012). The HORIZON study confirmed that HC2 performed as expected in a study assessing assay performance in primary screening populations. HORIZON evaluated unselected, consecutive primary cervical SurePath samples using QIAGEN HC2, Roche cobas® HPV Test, Hologic Gen-Probe APTIMA®, and Genomica CLART® assays (Preisler *et al*., 2013). HC2 testing demonstrated the highest overall positive and negative reproducibility. Low negative reproducibility results in false negatives that potentially compromise the negative predictive value of HPV screening. Low positive reproducibility leads to false positive results, possibly compromising the clinical efficacy of a screening programme. HC2 showed the best combination of positive and negative reproducibility. The HORIZON study also showed higher than expected positivity rates for PCR assays in a primary screening population. This could have implications for a screening programme's costs and efficiency, particularly if the evidence to implement HPV testing was based on data generated using HC2 technology (Preisler *et al*., 2013; Rebolj *et al*., 2014).

QIAGEN provides proven, integrated solutions offering fully clinically validated HC2 technology, tailored testing infrastructure, as well as partnership in education and training to reduce the clinical, economic, and societal burden imposed by cervical cancer. The Rapid Capture® System is a well-proven, highly reliable instrument solution. QIAGEN automated technological solutions provide a range of benefits in HPV testing. It offers the flexibility to provide laboratories with the HPV testing as a primary screen and cytology triage testing of samples collected in digene Specimen Transport Medium, PreservCyt Solution or SurePath Preservative fluid for low-, medium-, and high-throughput solutions and workflows.

Using the *digene* HC2 HPV DNA Test has a long experience in HPV screening. The *digene* HC2 HPV DNA Test received regulatory approval for triage more than 15 years ago and then received approval for primary screening more than 10 years ago. The clinical and scientific experts worldwide generated the clinical data required to support the move from cytology to HPV testing, based on data generated with HC2 for over 20 years.

The QIAsure Methylation Test

QIAGEN recently launches the QIAsure Methylation Test, a novel CE-marked molecular diagnostic test for use in differentiating patients' risk of developing cervical cancer.

QIAsure is a quantitative methylation specific PCR (qMSP) test that can help to clinicians to determine whether a hrHPV positive patient is at short-

term risk of developing cervical cancer. QIAsure detects the presence of biomarkers associated with cervical carcinoma and advanced transforming cervical intraepithelial neoplasia (CIN), to objectively discern passive HPV infections from ones that need immediate attention. QIAsure can be used to triage a positive hrHPV test on the same specimen used for the HPV test. It can also be used as a confirmatory test to an atypical squamous cells of undetermined significance (ASC-US) cytology result. QIAsure looks for methylation of host cell genes FAM19A4 and miR124-2 in cervical cells. Methylation of these genes indicates carcinogenic cell transformation and high short-term risk of developing cervical cancer; absence of methylation indicates low short-term risk of developing cervical cancer.

Abnormal patterns of DNA methylation have been implicated in various cancers, including cervical cancer where promoter hypermethylation of the tumour suppressor genes FAM19A4 and/or miR124-2 indicates the presence of precancer or cancer (Wilting et al., 2010; De Strooper et al., 2014a; De Strooper et al., 2014b; De Strooper *et al*., 2016a; De Strooper *et al*., 2016b – Bierkens *et al*., 2013; Luttmer *et al*., 2015; Steenbergen *et al*., 2014). QIAsure examines promoter hypermethylation in bisulfite-converted DNA isolated from cervical specimens using a multiplex real-time PCR test. Positive results correlate with the presence of carcinogenic cells and advanced transforming CIN lesions. In clinical trials, QIAsure testing was performed on physician-collected cervical specimens from 258 hrHPV-positive women including 117 without evidence of CIN 2 or worse after 18 onths follow-up (CIN≤1), 42 with CIN 2, 30 with CIN 3, 50 with squamous cell carcinoma, and 10 with adenocarcinoma. IAsure detected 100% of carcinomas (squamous cell carcinoma and adenocarcinoma) in these samples, but varied in detection of other grades of CIN, from 88.9% in CIN 3+ to lower sensitivity for CIN 1/2. FAM19A4 and miR124-2 methylation analysis specifically detects advanced transforming CIN lesions (Steenbergen *et al*., 2014). CIN 2/3 with low levels of FAM19A4 and miR124-2 methylation have low short-term progression risk for cancer. These patients can be managed by close surveillance rather than treated.

Independent research from leading cervical cancer scientists confirms the effective use of FAM19A4/miR124-2 methylation analysis for detection of cervical carcinomas and advanced CIN 2/3 lesions (De Strooper et al., 2014a; De Strooper et al., 2014b; De Strooper *et al*., 2016a; De Strooper *et al*., 2016b –Bierkens *et al*., 2013; Luttmer *et al*., 2015;). In a subgroup of women over 30 years (n=287), the CIN 3+ sensitivity and specificity for FAM19A4 methylation (88.3% and 62.1%) was greater than that of cytology (85.0% and 47.6%) and HPV 16/18 genotyping (70% and 57.7%) (Luttmer *et al*., 2015). DNA methylation analysis of FAM19A4/ miR124-2 has an equal

performance and high sensitivity for identifying high-grade CIN and cervical cancer in hrHPV positive brush- and lavage-collected self-samples.

The new QIAsure Methylation Test perfectly adds to QIAGEN's portfolio of diagnostic tools for use in women's health in primary screening.

QIAGEN's digene® HC2 HPV Test, the Gold Standard for sensitive, early detection of high-risk HPV, has been evaluated in clinical trials involving more than 1 million women – and proven through 15 years of clinical practice and more than 90 million tests worldwide. The digene HPV test uses advanced Hybrid Capture 2 technology to directly detect the presence of 18 types of HPV and is the only HPV assay that examines the entire length of the genome, which helps prevent false negatives caused by gene deletions that occur naturally during a woman's biological integration of the HPV virus. When the digene HPV Test rules out high-risk HPV, the result is highly reliable, based on the higher sensitivity of the HC2 technology. This benefit is seen to be of critical value in screening settings, in particular as intervals between screenings are extended as well as in primary screening settings. QIAGEN was the first company to implement large primary screening programs and has shown unrivalled validation and performance in doing so.

2. CONCLUSIONS

In conclusion, the HPV DNA testing used for the primary screening for prevention of cervical cancer must be clinically validated. Criteria for clinical validation exists from 2009 (Meijer *et al*., 2009) and were recently confirmed in 2015 (Mayrand *et al*., 2007). The *digene* HC2 HPV DNA Test is the most clinically validated assay. It detects more disease earlier allowing thus less severe treatment and it provides long duration of protection following a negative result. It is objective, standardised, quality assured and can be automated. It has been evaluated in clinical trials involving more than 1 million women – and proven through 15 years of clinical practice and more than 90 million tests worldwide. The digene HPV test uses advanced Hybrid Capture 2 technology to directly detect the presence of 18 types of HPV and is the only HPV assay that examines the entire length of the genome, which helps prevent false negatives caused by gene deletions that occur naturally during a woman's biological integration of the HPV virus. When the digene HPV Test rules out high-risk HPV, the result is highly reliable, based on the higher sensitivity of the HC2 technology. It can minimise the incidence of cervical cancer, and the morbidity and mortality it causes, even in low-resource settings. It can bring the cervical cancer free world.

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THE ALBANIAN EXPERIENCE IN CERVICAL CANCER SCREENING, PROBLEMS AND ACCOMPLISHMENTS

Majlinda IKONOMI
Oncology Service, University Hospital Center, Mother Theresa, Tirana, *Albania*
Shahin KADARE
Laboratory of Morphologic Diagnosis, Tirana, Albania

ABSTRACT

Check-ups and periodic health examinations aim prevention and early diagnosis in individuals without symptoms. There is lack of evidence for health check and most part of the criteria specified for screening are not met. Primary management of the cases discovered from the screening program, disease staging found during the screening program, post-surgical treatment of cases discovered from the screening program, follow-up of the target population and insurance from cancer, evaluation and interpreting of the results of the screening program are part of the performance indicators and their impact in the screening process. We analyzed the data collected during a Pilot screening study and the data from an opportunistic screening. These data have been dispersed according to age and cytology diagnosis and were compared between the two groups. In the opportunistic group it was calculated specificity, sensitivity and predictive value. The sensitivity and specificity in both groups were 92% and 90% respectively. The ASCUS/SIL rate was 4.46 in the organized group and 4.92 in the opportunistic group. Quality assurance for Pap-Smears is designed to minimize underdiagnosis in pathology and cytopathology. HPV rate in the ASCUS group was 60%. Conventional papsmear is a good diagnostic tool in both, opportunistic and organized system. HPV testing can serve as a triage for ASCUS group. The screening should be designed well in order to evaluate its performance and indicators.

***Keywords*:** CVC, Pap test, HPV test

1. INTRODUCTION

Screening of cancer in general is based on ***organized public programs*** like national screening programs for breast, colorectal, cervical cancer in the European Union or by ***Health checks (ref)***. Screening should allow the diagnosis of the disease at a stage when there is a possibility of healing or the

methods of treatment are not invasive. Check-ups and periodic health examinations aim prevention and early diagnosis in individuals without symptoms. There is lack of evidence for health check and most part of the criteria specified for screening are not met (Arbyn *et al*., 2004). This is the reason why not all campaigns of cancer screening have the same effectiveness (Aareleid *et al*., 1993).

Cytology, known as Papanicolaou (Pap) test for cervical cancer has demonstrated its efficacy in reducing specific cancer mortality for cervical cancer with its improved prognosis for patient diagnosed in latent period of the disease (Nygard *et al*., 2002). Pap test was first introduced in Albania in the late 1980s at the Oncology Service, University Hospital Centre, Tirana, Albania. The first attempts for screening have been made in this center as the first one involved in fight against cancer including cervical cancer. After this period Pap test has been used widely and played an important role in the early detection of cervical cancer. Its use has passed through opportunistic examination to pilot projects in several big cities of Albania, but still not as part of a national screening program. Several efforts have been made by different Albanian Health institutions and also international partners to influence the politic makers for including it as part of a population based screening program (ref). Pap test can be applied as a Conventional smear (CS) or a liquid based cytology (LBC). CS is widely used in our country for cervical cancer screening owing to its low cost and easy application (Hakama *et al*., 2008). Low preventive screening varies by region and contributes to poor outcomes for cervical cancer as it is still a high stage disease in our country (Raud and Klaar 2006). Several comparative urban and rural research on preventive screening has focused on government programs (EUROCHIP, 2010). The aim was to describe an organized pilot screening program and an opportunistic one to compare their performance indicators and to define requirements for the improvement of national implementation in the future.

2. MATERIALS AND METHODS

We used individual data from two independent screening centers. We analyzed the data collected during the Pilot screening study in 4 big cities of Albania in 2008 ***as an organized screening program***. Women were invited by the media to participate in the screening. The data were retrospectively collected by the archive of Laboratory of Morphologic Diagnosis (LDM). We also collected the data from the Pathologic anatomy Laboratory of Hygeia Hospital in 2013 as part of an ***opportunistic screening process in insured women.*** In this group the women had pap test as part of an insurance package of their health check-up

These data have been dispersed according to age and cytology diagnosis and compared for both groups.

In the opportunistic group we calculated ***specificity, sensitivity*** and ***predictive value.***

In the ***ASCUS*** category we calculated the sensibility in relation to the ***HPV testing*** (only offered in the opportunistic screening group). The samples were collected in both programs by gynecologist or midwives in a health center. The smears were prepared by conventional cytology, stained with Papanicolau method and interpreted by pathologist in three different laboratories according to the Bethesda System of reporting 2001. The cytological diagnosis of abnormal Pap tests were compared with the results of corresponding biopsies to calculate the sensitivity and specificity. Comparisons were performed using standard cross-table analysis.

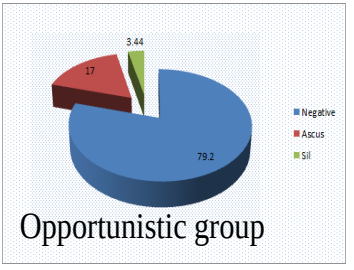
3. RESULTS

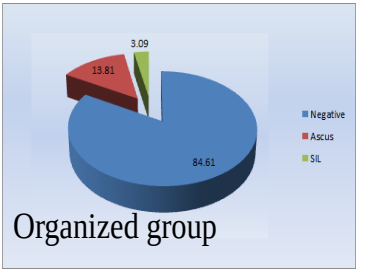
2991 women were involved as part of the organized screening project and 1860 as part of the opportunistic insured women. The results of each group are in the table 1 and 2 reported. There is no significant difference for the diagnostic categories of each group. The ASCUS category is higher in the opportunistic group than in the organized one as well as the positive cases. The dispersion of women examined according to age also has no significant difference. But however in the opportunistic group the group age that dominates is 31-40yrs old (37.6%) followed by 41-50 yrs (26.8%) while in the organized group there is 32.36% of the age group 41-50 yrs followed by 31.1% of the group age of 31-40 yrs old. ASCUS /SIL rate for each group is 4.46 for the organized and 4.92 for the opportunistic one.

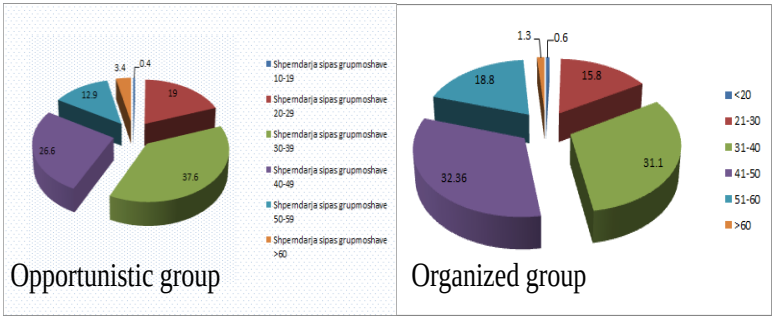
	<20 yo	21-30 yo	31-40 yo	41-50 yo	51-60 yo	>60	Total	
Women examined	20 (6,69%)	474(15,85)	932(31,16)	968(32,3)	562(18,78)	35(1,17)	2991	2991
No inflammation	3(3,64)	127(15,39)	215(26,06)	247(29,9)	218(26,4)	15(1,8)	825(27,58)	825(27,58)
Mild inflammation	8(7,48)	172(16,08)	348(32,5)	346(32,3)	183(17,1)	13(1,21)	1070(35,77)	1809(60,48)
Marked inflammation	5(7,86)	116(18,23)	222(34,9)	209(32,8)	80(12,57)	4(0,6)	636(21,26)	
Microorganisms	2(1,94)	26(25,2)	32(31,06)	37(35,9)	6(5,8)		103(3,44)	
ASCUS		28(9,9)	76(27,4)	87(30,96)	34(12,09)		281(9,39)	365(12,20)
ASCUS-R		6(18,7)	9(28,1)	13(40,6)	2(6,2)		32(1,06)	
ASCUS-H		3(5,7)	16(30,7)	23(44,2)	8(15,38)	2(3,8)	52(1,73)	
LSIL		10(13,3)	21(28)	28(37,3)	16(21,3)	2(2,6)	75(2,5)	75(2,5)
HSIL		3(18,75)	3(18,75)	7(43,7)	3(18,75)		16(0,53)	16(0,53)
AGUS			0 17(34,6)	23(46,9)	9(18,36)		49(1,63)	49(1,63)
Carcinoma			0	01(50,0)	1(50,0)		2(0,0668)	2(0,0668)
Inappropriate		12(15)	26(32,5)	22(27,5)	18(22,5)	2(2,5)	80(2,67)	80(2,67)

	10-19	20-29	30-39	40-49	50-59	>60
Negative	4	293	566	386	176	48
ASCUS	1	27	44	33	14	3
ASCUS R	0	27	46	35	17	3
ASCUS-H	0	0	3	3	4	0
SIL	0	15	26	12	8	4

In the organized group it was impossible to calculate the rate of ASCUS with HPV positivity as not offered this examination for triage. In the opportunistic group from 91 cases of ASCUS triaged with HPV 60 of them were positive. The organized pilot project included only the Pap test coverage and no follow up of cases with abnormalities and positive results. In this situation it was not possible to calculate sensitivity and specificity of the test taking as threshold the biopsy results. In the opportunistic group the sensitivity and specificity were 95.45% and 52.63% respectively (tab 3).

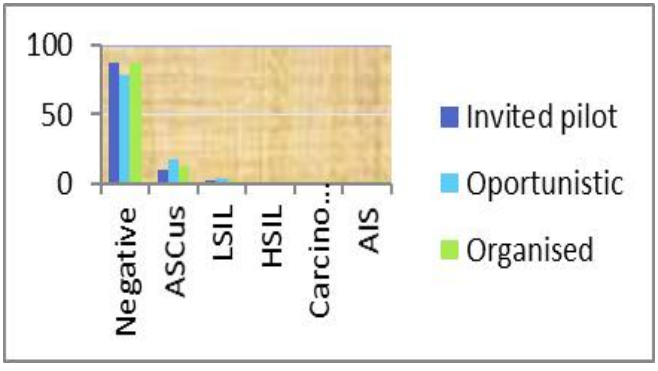




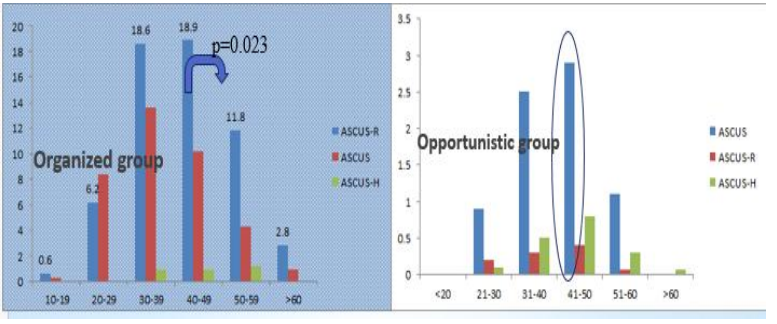


	ASCUS/SIL	HPV +/ASCUS
oportunistic	4.9	42%
organised	4.6	

	ASCUS	Negative	LSIL	
	73	9	9	91
HPV +	43	8	9	60
HPV-	30	1	0	31
				91



	CIN1	CIN2	Ca	NEG
ASCUS	5	1		4
AGUS	1			3
ASC-H	2	1		1
LSIL	5	2		1
HSIL		2		
Ca			3	
NEG			1	10



4. DISCUSSION

According to our data opportunistic screening was as likely to detect precancer and cancer as was organized screening. The overall similarity between the two types of screening is seen in tables 1,2 and 3, but multivariate analysis is required for a more informative quantitative comparison. The group of abnormal pap tests and positive ones was higher in the opportunistic group a thing that can be explained with the fact that in the organizing group it was just a smear taking process rather than an indication for routine examination in maternity wards and family planning clinic. Over screening is a more evident feature of the opportunistic screening in our setting (EC 2008).

Recommendations of the Council of the European Union straights out the fundamental principles of best practice in early detection of cancer. The EU member states have implemented national organized screening programs for three cancer sites (cervical, breast and colorectal cancer) with a well-informed, population based approach and with appropriate monitoring, and evaluation of quality assurance at all levels, taking into account European quality assurance guidelines for cancer screening (WHO 1986). In this contest reports of WHO for screening in developing countries with middle incomes

stress that central to the success of any screening program is functioning in its entirety (Arbyn *et al*., 2010). The pilot project organized in our country as an organized screening program is "the lesson to be learned". This project has not been well thought and implemented as long as it didn't included all the steps to be followed for quality control and follow up. If we see in the opportunistic group the fact that all the process is within one structure with gynecologist, laboratory of cytology, central informatization of patients results, follow up with biopsy and HPV testing or colposcopy for abnormal cases, permits the control of the system , the measurement of the indicators of performance like sensitivity, sensibility, and the points to be improved. In the organized group it was unable to calculate sensitivity, specificity and ASCUS/HPV rate because the abnormal cases were lost and not followed up. Studies from developing countries have showed that the quality of cytological examinations has been a barrier to successful screening. A study of 13 cytology centers from developing countries has faced problems related to poor quality of the service, not trained personnel, false -negativity rate of 54% (Arbyn *et al*., 2010; Laara *et al*., 1987). In developing countries, creating systems that ensure the Quality for Cytological Exams is still a challenge (Laara *et al*., 1987; Kunst and Mackenbach 1994). In both of our groups the ASCUs/SIL rate is higher than guidelines reports. This can be explained with the experience, with the fear of losing cases with abnormalities, with the category itself and with the fact that in our institutions the control of quality control is not a well established program. It is generally agreed that cytology screening for cancer of the cervix has been effective in reducing the incidence and mortality from the disease in many developed countries. It is the organised programmes that have shown the greatest effect, while using less resources than the unorganised programmes. There is general agreement that high quality cytology is a highly specific screening test, with estimates of the order of 98-99%. There is less agreement on the sensitivity of the test, cross-sectional studies have suggested sensitivity in the order of 50% in some circumstances. However, studies that have been able to assess sensitivity longitudinally have produced estimates that approximate to 75% (Spayne *et al*., 2008; Schiaffino *et al*., 2003). Molecular and epidemiological studies have unequivocally shown that the vast majority of cervical cancer cases worldwide are caused by persistent infections with some high-risk types of the human papillomavirus family. From the perspective of defining preventive strategies, the HPV-attributable fraction should be considered to be 100%. In triage studies (investigations of the minor abnormalities detected by cytology) and in screening studies (when both cytology and HPV tests are jointly performed) the cross-sectional sensitivity of the HPV test to detect HSIL or more advanced lesions is at least as good as cytology. In most studies, the

reported sensitivity of the HPV test is some 10% higher than cytology (ESCA 2006; van Ballegooijen *et al*., 2000).

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PREVENTION, DIAGNOSIS AND TREATMENT OF CERVICAL CANCER IN MONTENEGRO

Đurđica OSTOJIĆ
Public Health Institute of Montenegro, Department for Control and Prevention of Non-Communicable Diseases

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ABSTRACT

The cervical cancer is the disease that can be successfully prevented because it has a long preinvasive period and it can be effectively controlled by screening. The treatment of preinvasive lesions and early stage of the disease is highly successful. According to the Montenegro National Cancer Control Plan, Podgorica, July 2011 and National Program for early cervical cancer detection, Podgorica, September 2011, the target population are women aged 25-64 years. The main aims of the Montenegro National Cancer Control Plan are: improvement of the efficiency and cancer control through data collection and surveillance, reduction in cancer incidence through primary prevention measures, to ensure early detection and screening program aimed at reducing the cancer-related incidence and mortality, improvement of organization and services for diagnostics and treatment of malignant diseases, aimed at reducing the morbidity and mortality. Improving the quality of life of cancer patients and their family through the establishment of palliative care and rehabilitation services, quality improvement and strengthening human resources and education.Program for early cervical cancer detection in Montenegro started in Podgorica municipality from the 18th of July 2016. The target group contains 7.555 women aged 30-34 years and the duration of one cycle of screening is five years, according to the Comprehensive cervical cancer control: a guide to essential practice - 2nd edition, World Health Organization. Coverage: 34,55% of the total number of women aged 30-34 in Montenegro. Screening test: Abbott Real Time PCR HPV (human papilloma virus) as the primary test is based on detection of DNA of high-risk HPV genotypes. All HPV positive women are refered to do LBC test of the cervical smears and colposcopy to detect the existence of precursors of cervical cancer. Screening invitation scheme: organized and centralized. Each step of the Programme is IT supported as a part of Integrated informatical health care system in Montenegro. Gynecological examinations, sampling smears of the cervix uteri, colposcopy, biopsy, additional tests and treatment that are organized in the Cervical Cancer Screening Programme are free of charge for the participating women.

Keywords: CVC treatment, Montenegro

1. INTRODUCTION

The cervical cancer is the disease that can be successfully prevented because it has a long preinvasive period and it can be effectively controlled by screening. The treatment of preinvasive lesions and early stage of the disease is highly successful.

The main aims of the Montenegro National Cancer Control Plan and National Program for early cervical cancer detection are: improvement of the efficiency and cancer control through data collection and surveillance, reduction in cancer incidence through primary prevention measures, to ensure early detection and screening program aimed at reducing the cancer- related incidence and mortality, improvement of organization and services for diagnostics and treatment of malignant diseases, aimed at reducing the morbidity and mortality. Improving the quality of life of cancer patients and their family through the establishment of palliative care and rehabilitation services, quality improvement and strengthening human resources and education.

According to the Montenegro National Cancer Control Plan and National Program for early cervical cancer detection, the target population is women aged 25-64 years.

Program for early cervical cancer detection in Montenegro started in Podgorica municipality from the 18th of July 2016.

The target group involves 7.555 women aged 30-34 years old. The duration of one cycle of screening is five years, according to the European Guidelines for Quality Assurance in Cervical Cancer Screening, 2nd ed. 2008 and Comprehensive Cervical Cancer Control: a guide to essential practice - 2nd edition, World Health Organization. Coverage is 34,55% of the total number of women aged 30-34 in Montenegro.

2. MATERIALS AND METHODS

Abbott Molecular's *m*2000*sp* for sample preparation and the *m*2000*rt* for real-time amplification and detection are the cornerstones for this process.

Abbott Real Time PCR HPV (human papilloma virus) as the primary test is based on detection of DNA of high-risk HPV genotypes. All HPV positive women are referred to do LBC test of the cervical smears and colposcopy to detect the existence of precursors of cervical cancer. In cervical cancer screening ThinPrep® (Hologic) LBC test is in use.

Screening invitation scheme: organized and centralized. Each step of the Programme is IT supported as a part of Integrated Informatical health care system in Montenegro.

Gynecological examinations, sampling smears of the cervix uteri, colposcopy, biopsy, additional tests and treatment that are organized in the Cervical Cancer Screening Programme are free of charge for the participating women.

Therefore, as stated before, the target group involved 7.555 women aged 30-34 years, registered by chosen doctors for women/gynecologists, in primary health care.

With the algorithm bellow we will try to explain the way how screening program for early cervical cancer detection works, step by step.

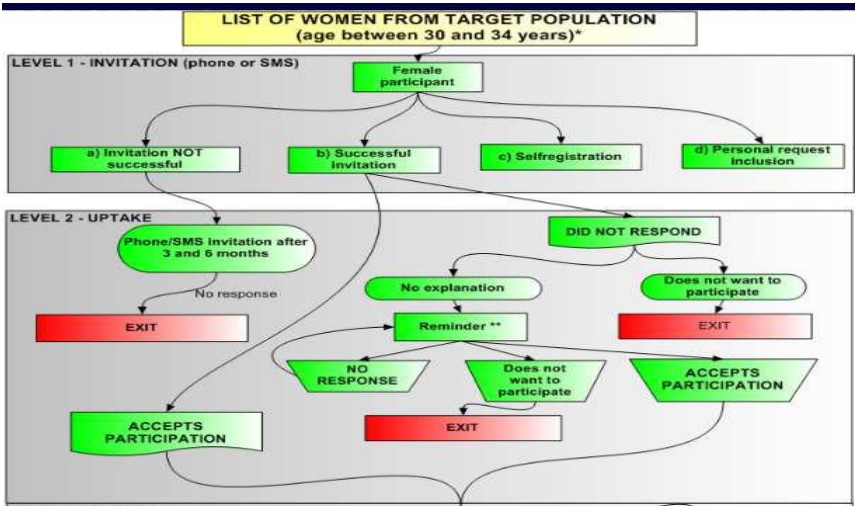


Fig. 1: Level 1 and 2 (invitation and uptake) of screening program for early cervical cancer detection.

Female participants should be invited by phone or SMS, self-registered or included by personal request.

If invitation is not successful, phone or SMS invitation is repeated two times, after 3 and 6 months and if there is no response, participant exits the screening program.

If invitation is successful and woman accepts participation, she has to visit the chosen doctor for women/gynecologist where she has to fill out two questionnaires.

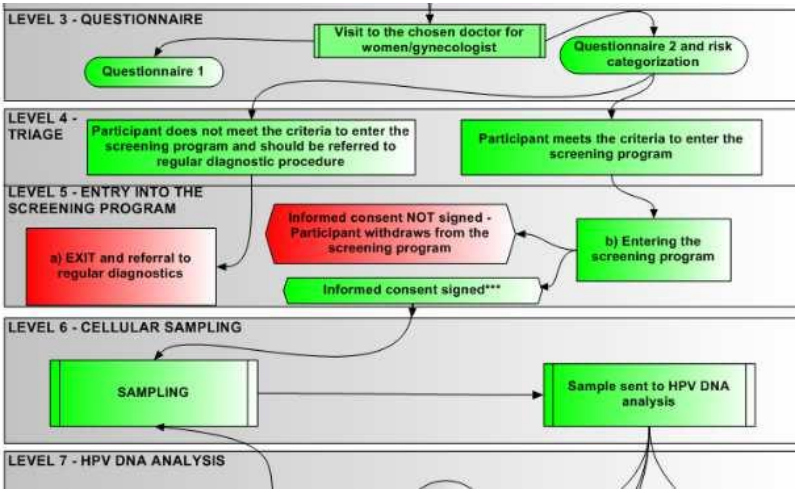


Fig. 2: Level 3,4,5 and 6 (Questionnaire, triage, entry into the screening program and sampling) of screening program for early cervical cancer detection.

Questionnaire 1 contains general information about participant, while questionnaire 2 contains questions about possible existing symptoms of cervical cancer and family history.

If participant give any positive answer in questionnaire 2, she does not meet the criteria to enter the screening program, exits the screening program and should be referred to regular diagnostic procedure. Early detection of the cervical cancer by means of rapid identification of the first symptoms is integrated into primary health care services and there is a clearly defined referral system from primary care to secondary / tertiary care for suspect cases and early diagnosis.

If participant does meet the criteria to enter the screening program, she signs informed consent. If informed consent is not signed, participant exits from the screening program.

Next step is sampling. Samples are sent to HPV DNA analysis in the Public Health Institute.

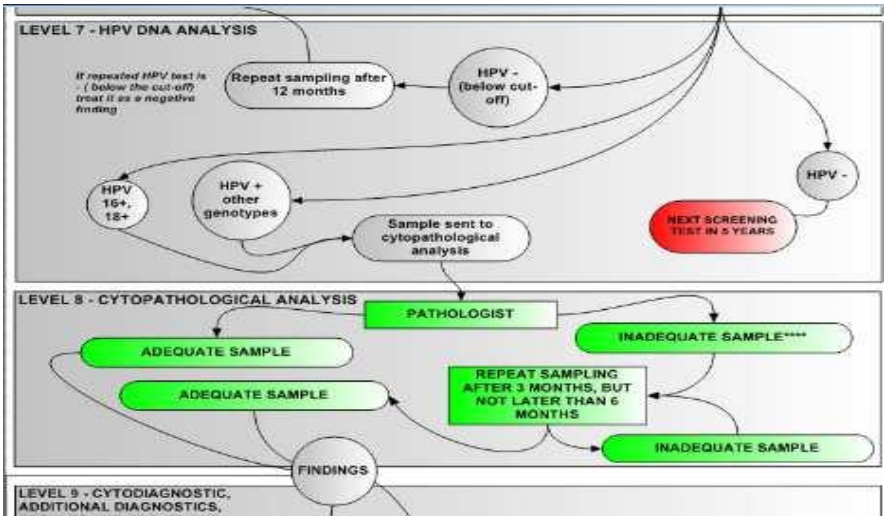


Fig. 3: Level 7 and 8 (HPV DNA analysis and cytopathological analysis) of screening program for early cervical cancer detection.

If the result of HPV DNA analysis is negative, participant should repeat next screening test after 5 years.

Abbott RealTime HR HPV test enables detection of DNA of Human papilloma virus and below the "cut-off" defined by the manufacturer. It should not be ignored the probability that this finding was caused by inadequate sampling (not sampled a sufficient number of cells, no data about the course of infection in the period when sampling is performed, etc).

If the result of HPV DNA analysis is negative - bellow cut off, participant should repeat sampling after 12 months. If repeated HPV test is bellow the cut off, we treat it as a negative finding.

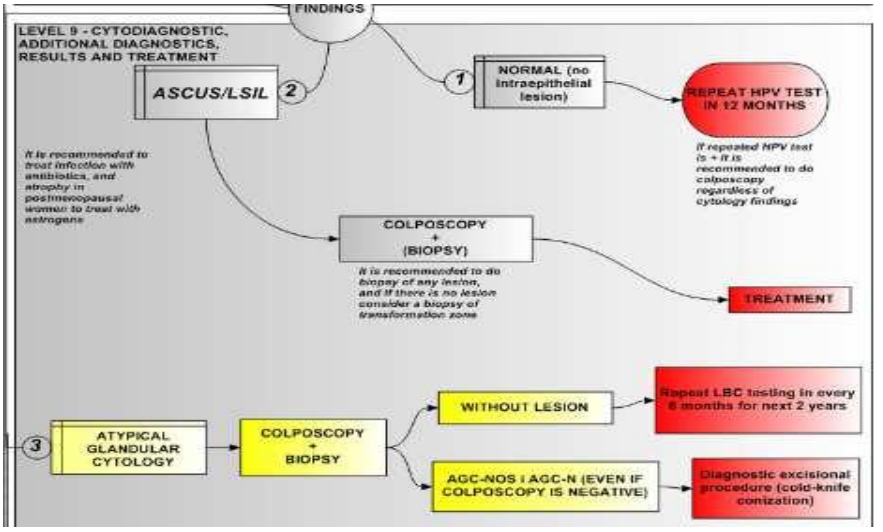
HPV-positive women are re-invited after three months for LBC (cytopathological analysis). If the result of HPV DNA analysis is positive samples are sent to cytopathological analysis.

Positive results can be: 16+, 18+, other genotypes + (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 i 68), 16+ and 18+, 16+ nad other genotypes +, 18+ and other genotypes +, 16+ and 18+ and other genotypes +.

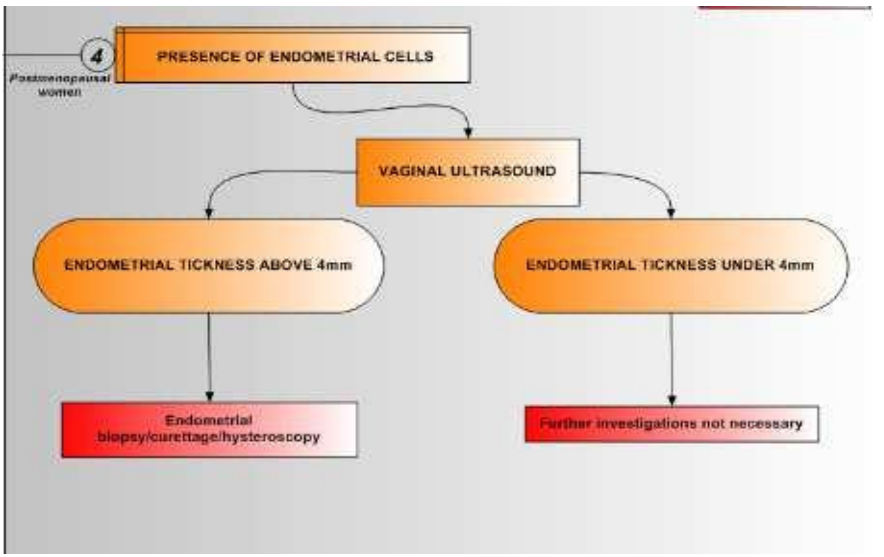
Cythopathological analysis is done by pathologist who decides which sample is adequate and which is not.

Sample can be inadequate for many reasons such as less than 8.000 cells, more than 75% of undetermined cells (blood, inflammation), broken slides and incomplete or missing identification data about participants.

If sample is inadequate, it is indicated to repeat sampling after 3 months, but not later than 6 months.



Picture 4: Level 9 (Cytodiagnostic, additional diagnostics, results and treatment) of screening program for early cervical cancer detection



Picture 5: Level 9 (Cytodiagnostic, additional diagnostics, results and treatment) of screening program for early cervical cancer detection

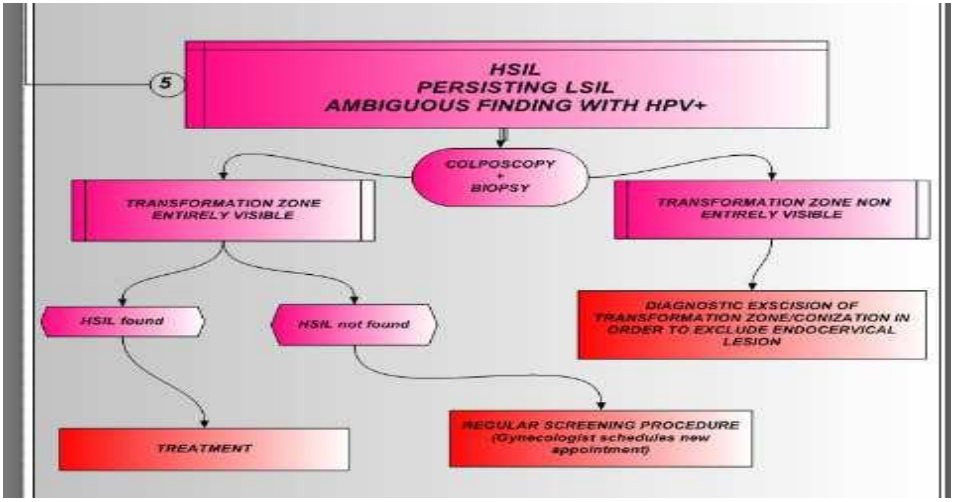


Fig. 6: Level 9 (Cytodiagnostic, additional diagnostics, results and treatment) of screening program for early cervical cancer detection.

Bethesda (2001) said that based on Cervical Cytology Classification, results can be: i) **normal (no intraepithelial lesion)**. Participant should repeat HPV test in 12 months. If repeated HPV test is positive, it is recommended to do colposcopy regardless of cytology findings, ii) **ASCUS/LSIL,** if the result is ASCUS/LSIL, colposcopy is indicated, and eventually biopsy and adequate treatment if it is necessary. It is recommended to do biopsy of any lesion, and if there is no lesion consider a biopsy of transformation zone, iii) **atypical glandular cytology.** If the result is Atypical Glandular Cytology, it is indicated to do colposcopy and biopsy. If there is no lesion, it is indicated to repeat LBC testing in every 6 months for next 2 years.If there is AGC-NOS and AGC-N (even if colposcopy is negative), it is indicated to do diagnostic excisional procedure (cold-knife conization), iv) **presence of endometrial cells in samples of postmenopausal women** Vaginal ultrasound is indicated. If the endometrial thickness is above 4 mm, biopsy, curretage and hysteroscopy are indicated. If the endometrial thickness is under 4 mm, further investigations are not necessary and, v) **HSIL, persisting LSIL, ambiguous finding with HPV +.** Colposcopy and biopsy are indicated. If transformation zone is entirely visible and HSIL is found, treatment is necessary. If transformation zone is entirely visible and HSIL is not found, participant is reffered back to regular screening procedure (gynecologist shedules new appointment). If transformation zone is not entirely visible, diagnostic excision of transformation zone/conization

is indicated in order to exclude endocervical lesion.

3 . RESULTS

At this moment, we are not able to show off the final results because of short implementation period of the National program for early cervical cancer detection, but we highly hope that we will be after a year.

4. DISCUSSION

A lot of women, unfortunately, visit a gynecologist for the first time when the disease is already symptomatic or in advanced stage and when the treatment is difficult and uncertain. Therefore, the most important part of the fight against cervical cancer is implementation of organized screening. Main goal of program for early cervical cancer detection is to reduce the number of cases and number of deaths from this disease. Besides that, early detection and successful treatment significantly improves life quality, preserve fertility and highly reduces treatment costs. In countries with well-organized population screening, number of women suffering and dying from cervical cancer is significantly reduced. Significant decrease in the incidence and mortality in some countries such as England, Finland and Iceland is closely related to the quality of organized screening. The best example is Finland who conducts organized screening program for 45 years and where the mortality rate is reduced by 80% during this time.

We hope that we will be able to achieve similar results in the future and significantly raise women awareness of importance of preventive examinations.

ACKNOWLEDGMENTS

I thank the members of the working group that was formed by the Ministry of Health of Montenegro who have made significant contributions to the preparation of the algorithm as well as in the implementation of the Program of early cervical cancer detection.

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MACEDONIAN EXPERIENCE RELATED RESULTS FROM NATIONAL PROGRAM FOR CERVICAL CANCER SCREENING

Elena KJOSEVSKA
Institute of Public Health (IPH), Skopje, Republic of Macedonia
Aziz POLOZHANI
University, Mother Teresa, (UMT) Skopje, Macedonia
Silvana ONCHEVA, Shaban MEMETI
Institute of Public Health (IPH), Skopje, Republic of Macedonia
Jovanka KOSTOVSKA and Sanja SAZDOVSKA
Ministry of Health (MoH), Skopje, Republic of Macedonia

ABSTRACT

Worldwide, the cervical cancer (CC) is the 4th most common form of cancer among women, as well as 4th cause of death in women. There were 528,000 cases of CC detected in 2012. In the same year there were 266,000 deaths. Over 80% of CC is detected in developing countries. The American Cancer Society offers the following list of risk factors: infection with human papilloma virus (HPV), HIV, chlamydia, stress and stress related disorders, diet, smoking, multiple pregnancies, exposure to the hormonal agent diethylstilbestrol (DES), family history of CC, etc. Vaginal bleeding can be linked with CC, but symptoms may be absent until the moment when the cancer has not come at an advanced stage. The first recognizable symptoms of the disease include: watery or bloody discharge from vagina, heavy and unpleasant odor, bleeding from vagina during and after sexual intercourse between two menstruation or during menopause, periods might be heavier and last longer than normal, moderate pain during sexual intercourse. For premalignant dysplastic changes, CIN (cervical intraepithelial neoplasia) grading is used. This term classifies light dysplasia as CIN1, moderate dysplasia as CIN2, and severe dysplasia as CIN3 (CIS).The aim of the paper is to present the situation with CC morbidity and mortality in the RM and the effects of preventive measures after the introduction of a screening program for CC early detection in 2012.Using retrospective method of work, the

statistical data from the monitoring of CC morbidity and mortality in the world and in Macedonia has been analyzed, as well as an overview of government annual programs and annual reports of the IPH related to the results of screening for early CC detection in Macedonia. Measures and activities designated in specific strategies and action plans are presented. Published materials from civil society sector are also used in the context of improving the quality of screening and providing larger and better coverage of women with this program.In Macedonia, the incidence rate or newly registered cases of CC is around 22.5 per 100.000 inhabitants. Death rates of CC is declining (from 6.5 in the year 2000 to 3.8 per 100,000 women registered in 2015).
***Keywords*:** CVC, death rate, women, CIN, CIN1, CIN2 and CIN3, Republic of Macedonia

INTRODUCTION

Worldwide, the cervical cancer (CC) is the 4th most common form of cancer among women, as well as 4th cause of death in women. There were 528,000 cases of CC detected in 2012. In the same year there were 266,000 deaths. Over 80% of CC is detected in developing countries. The American Cancer Society offers the following list of risk factors: infection with human papilloma virus (HPV), HIV, chlamydia, stress and stress related disorders, diet, smoking, multiple pregnancies, exposure to the hormonal agent diethylstilbestrol (DES), family history of CC, etc. Vaginal bleeding can be linked with CC, but symptoms may be absent until the moment when the cancer has not come at an advanced stage. The first recognizable symptoms of the disease include: watery or bloody discharge from vagina, heavy and unpleasant odor, bleeding from vagina during and after sexual intercourse between two menstruation or during menopause, periods might be heavier and last longer than normal, moderate pain during sexual intercourse. For premalignant dysplastic changes, CIN (cervical intraepithelial neoplasia) grading is used. This term classifies light dysplasia as CIN1, moderate dysplasia as CIN2, and severe dysplasia as CIN3 (CIS).

The aim of paper

The aim of the paper is to present the situation with CC morbidity and mortality in the RM and the effects of preventive measures after the introduction of a screening program for CC early detection in 2012.

MATERIAL AND METHOD

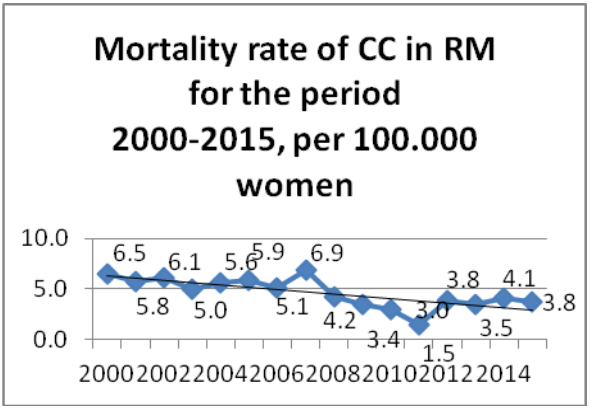
Using retrospective method of work, the statistical data from the monitoring of CC morbidity and mortality in the world and in Macedonia has been analyzed, as well as an overview of government annual programs and annual reports of the IPH related to the results of screening for early CC detection in Macedonia. Measures and activities designated in specific strategies and action plans are presented. Published materials from civil society sector are also used in the context of improving the quality of screening and providing larger and better coverage of women with this program.

RESULTS AND DISCUSSION

In Macedonia, the incidence rate or newly registered cases of CC is around 22.5 per 100.000 inhabitants.

Years	Number	Rate /100 000
2001	**387**	**38.0**
2002	**210**	**20.9**
2003	**248**	**24.6**
2004	**226**	**22.3**
2005	**221**	**21.8**
2006	**263**	**25.9**
2007	**218**	**21.4**
2008*	**222**	**21.7**
2009*	**226**	**22.1**
2010*	**230**	**22.4**
2010/2001	**59,4%**	**-**

Death rates of CC is declining (from 6.5 in the year 2000 to 3.8 per 100,000 women registered in 2015).



Year	Total deaths from CC
2008	43
2009	35
2010	31
2011	15
2012	39
2013	36
2014	42
2015	39

Diagram 1 and Table 2.Number of deaths from Cervical cancer Source: State Statistical Office, Skopje, Macedonia, Processed by Institute of Public Health, Skopje, Macedonia

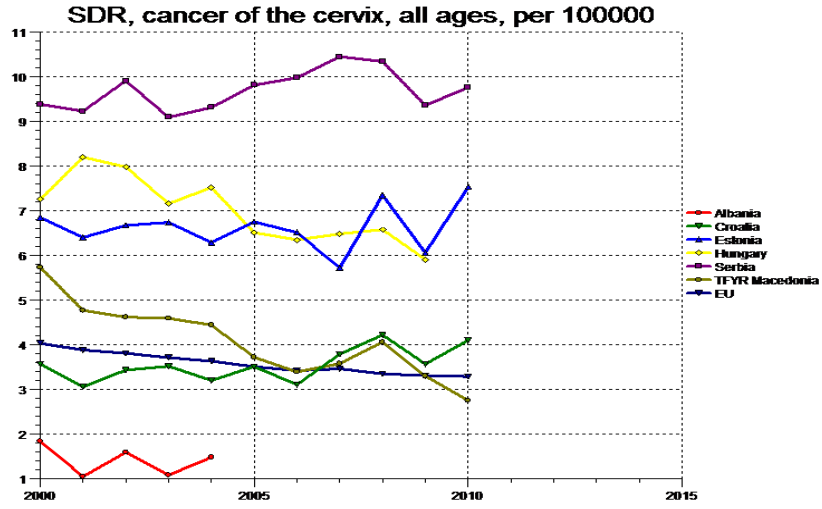


Diagram 2. Source: WHO. Health for All Data Base, 2015

PREVENTION OF CERVICAL CANCER

Cervical cancer can be prevented through the application and implementation of the following preventive measures:

1. Changing bad habits among the adult population that are associated with the risk of these diseases (inadequate diet, smoking, physical inactivity, unprotected sex) and reducing risk factors by limiting sexual partners and unprotected sex.
2. Screening, early detection through regular PAP - tests and other diagnostic tests
3. Use of HPV vaccination.

In order to reduce the incidence and mortality of women from CC in the country through preventive actions at the institutional level, the Government each year adopts Programme for early detection of malignant diseases in the Republic of Macedonia drafted by the Ministry of Health, as well as the program for early detection and prevention of CC.

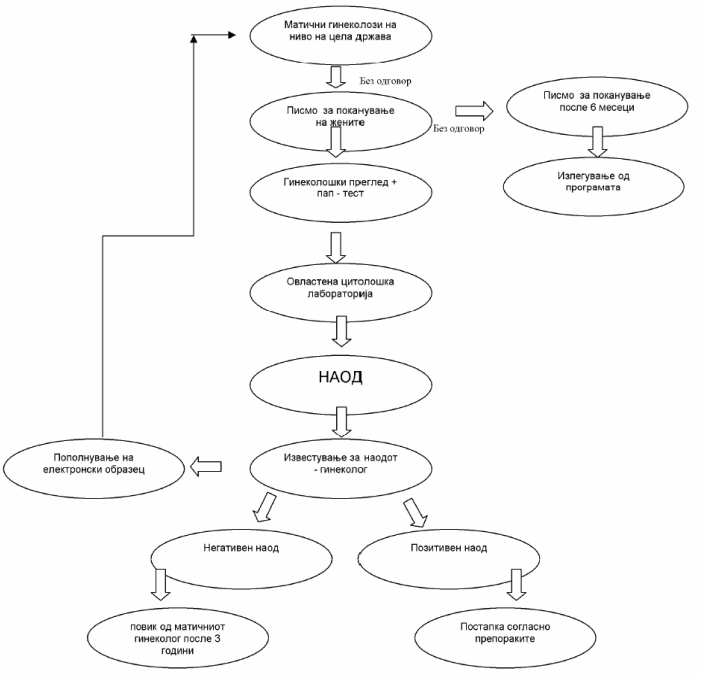
The activities are implemented by the Ministry of Health as coordinator, the gynecologists in primary health care, cytologists, histo-patologist's at the University Clinic of Gynecology and Obstetrics and private laboratories, 10 Public Health Centers that collected data on monthly basis from the gynecologists and patohistologist's, and the Institute for Public Health which collects data from 10 CPH on quarterly basis, analyze, prepare quarterly reports and submit them to the Ministry of Health.

History of the Cervical Cancer Screening Program

In 2009, the implementation of the Program for screening of cancer of reproductive organs started has been initiated in 4 municipalities as pilot communities and was more like a kind of campaign designed to raise awareness among women about regular gynecological examination and regular screening for early detection of CC.

Organised cancer screening for CC in the country with a free of charge PAP test has been introduced in 2012. According doctrinal views, women aged 24-60 years are targeted. Rrecommended interval for organized screening is 3 years and the first year covered women aged 24-35, and those aged 36-60 years who did not make a PAP test over the previous year. The second year, priority included women aged 36-45 years and the third year, women aged 46-60 years.

Scheme: Organizational algorithm of the Screening Program for Cervical Cancer



Source: Ministry of Health of the Republic of Macedonia, Skopje

Out of 48226 of the invited women in 2015, 81.8% of women aged 24-35 years and 18.2% of women aged 36-60 years during 2014 did the PAP test. The percentage of women who received an invitation out of invitees in all public health centers is satisfactory- 97.2%. Out of the total of 29887 examined women who made PAP test, 89.2% or 26668 of cytological smears are analyzed and 2.9% is the percentage of epithelial cell abnormalities detected by cytological smears analyzed. The number of PAP tests made, or the number of examined women in organized screening in the country is increasing. In 2012, 17595 Pap tests were made, and in 2015 29887 PAP tests which means growth in the range of 69,86% is recorded.

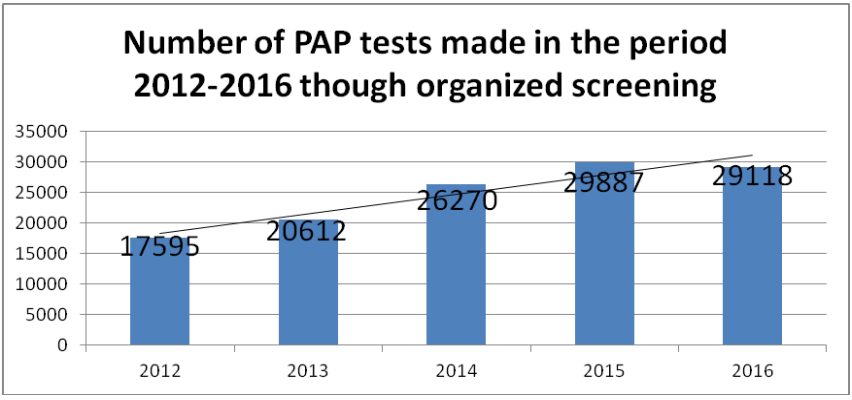


Diagram 3. Source: Institute for Public Health. Skopje, Republic of Macedonia, 2016

In most of the cases, HPV and CIN1 were detected.

Table 3. Detected pre-cancer lesions and cancer in Republic of Macedonia in 2016

36-45 години	36-45 години	Јануар	Феврyа	Март	Јануари-мар	Април	Мај	Јуни	Април-јуни	Јули	Август	Септем
Битола	негативен	53	110	66	229	190	62	71	323	108	147	42
Битола	ASC-US	1	2	2	5	2	2	4	8	1	2	3
Битола	ASC-H	0	0	0	0	0	0	0	0	0	1	0
Битола	HPV	0	5	3	8	7	3	1	11	1	7	4
Битола	CIN 1	3	3	6	12	5	9	4	18	6	6	6
Битола	CIN 2	0	0	0	0	0	0	0	0	1	0	1
Битола	CIN 3	0	0	0	0	0	0	11	11	0	0	0
Битола	CIS	0	0	0	0	0	0	0	0	0	0	0
Битола	Плочест карцином суспектен за инвазија	0	0	0	0	0	0	0	0	0	0	0
Битола	Инвазивен плочест карцином	0	0	0	0	0	0	0	0	0	0	0
Битола	AGC	0	0	0	0	0	0	0	0	0	0	0
Битола	Аденокарцином ин ситу	0	0	0	0	0	0	0	0	0	0	0
Битола	Цервикален аденокарцином	0	0	0	0	0	0	0	0	0	0	0
Битола	Аденокарцином неспецифициран поинаку NOS	0	0	0	0	0	0	0	0	0	0	0
Битола	Друга малигна неоплазма	0	0	0	0	0	0	0	0	0	0	0
Битола	вкупно абнормални	4	10	11	25	14	14	20	48	9	16	14
Битола	вкупен број	57	120	77	254	204	76	91	371	117	163	56
Битола	% на негативни тестови	93.0%	91.7%	85.7%		93.1%	81.6%	78.0%		92.3%	90.2%	75.0%
Битола	% на позитивни тестови	7.0%	8.3%	14.3%		6.9%	18.4%	22.0%		7.7%	9.8%	25.0%

36-45												
ЦЈЗ:		Јануари	Февруари	Март	Јануари-март	Април	Мај	Јуни	Април-јуни	Јули	Август	Септември
Гевгелија	негативен	12	112	108	232	67	91	87	245	41	28	92
Гевгелија	ASC-US	1	3	4	8	1	12	10	23	5	4	9
Гевгелија	ASC-H	0	0	0	0	0	0	0	0	0	0	0
Гевгелија	HPV	2	10	17	29	7	7	3	17	7	10	10
Гевгелија	CIN 1	0	4	4	8	2	1	2	5	5	2	3
Гевгелија	CIN 2	0	0	2	2	1	0	0	1	1	0	1
Гевгелија	CIN 3	0	0	0	0	0	0	0	0	0	0	0
Гевгелија	CIS	0	0	0	0	1	0	0	1	0	0	0
Гевгелија	Плочест карцином суспектен за и	0	0	0	0	0	0	0	0	0	0	0
Гевгелија	Инвазивен плочест карцином	0	1	0	1	0	0	0	0	0	0	0
Гевгелија	AGC	0	0	0	0	0	0	0	0	0	0	0
Гевгелија	Аденокарцином ин ситу	0	0	0	0	0	0	0	0	0	0	0
Гевгелија	Цервикален аденокарцином	0	0	0	0	0	0	0	0	0	0	0
Гевгелија	Аденокарцином неспецифицира	0	0	0	0	0	0	0	0	0	0	0
Гевгелија	Друга малигна неоплазма	0	0	0	0	0	0	0	0	0	0	0
Гевгелија	вкупно абнормални	3	18	27	48	12	20	15	47	18	16	23
Гевгелија	вкупен број	15	130	135	280	79	111	102	292	59	44	115
Гевгелија	% на негативни тестови	80.0%	86.2%	80.0%	82.9%	84.8%	82.0%	85.3%	83.9%	69.5%	63.6%	80.0%
Гевгелија	% на позитивни тестови	20.0%	13.8%	20.0%	17.1%	15.2%	18.0%	14.7%	16.1%	30.5%	36.4%	20.0%

Тетово/ПЕ Гостивар		Јануари	Февруари	Март	Јануари-март	Април	Мај	Јуни	Април-јуни	Јули	Август	Септември
Гостивар	негативен	121	93	94	308	78	85	31	194	76	71	63
Гостивар	ASC-US	0	0	0	0	0	0	0	0	1	0	0
Гостивар	ASC-H	0	0	0	0	1	0	0	1	0	1	0
Гостивар	HPV	2	0	0	2	1	0	0	1	0	0	0
Гостивар	CIN 1	0	1	0	1	0	0	0	0	0	0	0
Гостивар	CIN 2	0	0	0	0	0	0	0	0	0	0	0
Гостивар	CIN 3	0	0	0	0	0	0	0	0	0	0	0
Гостивар	CIS	0	0	0	0	0	0	0	0	0	0	0
Гостивар	Плочест карцином суспектен за и	0	0	0	0	0	0	0	0	0	0	0
Гостивар	Инвазивен плочест карцином	0	0	0	0	0	0	0	0	0	0	0
Гостивар	AGC	0	0	0	0	0	0	0	0	0	0	0
Гостивар	Аденокарцином ин ситу	0	0	0	0	0	0	0	0	0	0	0

Source: Institute for Public Health. Skopje, Republic of Macedonia, 2016

A decision of the Minister of Health has been made on 05.10.2009, and the HPV vaccine for girls aged 12 years has been introduced. Silgard HPV vaccine is for prevention of the types 6, 11, 16 и 18. It is 98% effective and is approved by FDA (American administration for food and medicines) on 8th of June 2006. Silgard vaccine is also approved in EU. This vaccine was made compulsory in 2010. In 2017. gynecologists and laboratories contracted by the

Health Insurance fund are beneficiaries, and women aged 46-60 years and women from 36-45 who did not conduct PAP test in 2016 will be targeted.

A Working group within the Ministry of Health has been constituted to prepare an action plan for successful implementation of the program in the coming period. Future activities will be: i) strengthen the system of inviting women, increasing the coverage of Roma and other marginalized women, ii) introduction of a CC screening registry,, iii) introduction of new screening methods (HPV testing), iv) better education of women rights arising from the program, v) better and continued funding and, vi) increasing the number of gynecologists and cytologists, etc.

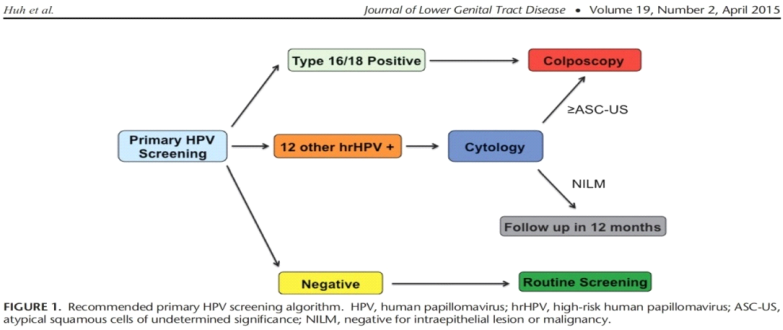


FIGURE 1. Recommended primary HPV screening algorithm. HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; ASC-US, atypical squamous cells of undetermined significance; NILM, negative for intraepithelial lesion or malignancy.

CONCLUSIONS

The increase in the coverage with organized CC screening in Macedonia for the period 2012-2015 is related to the greater awareness of women about the importance of screening to prevent their health, and this is due to preventive activities successfully implemented by the Ministry of Health. There are more and more precancerous changes at early-stage detected, which are further appropriately treated. It could result in successful treatment, rescued and prolonged life.

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THE ROLE OF PRIMARY HEALTH CARE AND OF THE OBSTETRIC - GYNECOLOGIST SPECIALIST IN THE PREVENTION OF THE CERVICAL CANCER

Mirela RISTA
University of Medicine of Tirana, University Hospital of Obstetrics-Gynecology, Koço Gliozheni, Tirana, Albania

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ABSTRACT

The implementation of the prevention programs for cervical cancer is now a task for the health care staff in the PHC. The guidelines and the protocols have been approved by Ministry of Health and will offer the same standard for everyone. They are based on the best knowledge and practices in the early detection of cervical cancer. The screening test is recommended for every woman at age 30 – 49 years old. All the women with a positive result need to be treated. The obstetric – gynecologist physicians specialized in colposcopy should diagnose and treat further these patients. We hope for the near future, to prevent the advances stages of cervical cancer in Albania.

Keywords: cervical cancer, screening program, colposcopy, HPV test, Pap smear

1. INTRODUCTION

The cervical cancer screening is one of the most effectiveness program among all screening programs in reducing the mortality. In Albania, about the half of the new cases with cervical cancer are diagnosed in advances stages of diseases, when the possibility for treatment is very low. The objective is to decrease the cervical cancer incidence rates through implementation of the periodic and organized screening test for the Albanian women. The national screening program for early detection of cervical cancer is now a priority of our government.

2. MATERIALS AND METHODS

Test application in the women of the 30-49 age-group.

The design of the guidelines for the screening that will be implemented by the primary healthcare service personnel.

The education and training of the primary healthcare (PHC) service personnel related to the knowledge for the evaluation stages of the Cervical Cancer and for the interpretation of each result identified in the screening test.

Practical workshops for the correct way on how to take the test with the personnel that will offer this service.

Training of more specialized Obstetric - Gynecologist physicians related to the colposcopy for the evaluation of the patients who have been resulted with an abnormal screening test.

3. RESULTS

Diagnosis, management, and follow-up of pre-invasive cervical lesions are now a major public health challenge. Cytology screening has been very successful in lowering cancer incidence and mortality in countries where excellent quality screening is available, yet false-positive results are common. The recommended algorithm for the Pap Smear test is found in table A at Appendices 1.

To have a wider coverage of the population that will be tested, based as well on the WHO guidelines, it was decided that the HPV screening test to be applied once per 5 years in the targeted age-group. Hence this application period for the re-testing has been evaluated as enough for having one curable disease stage i.e. pre-cancerous, even if one patient has positive results compared with another with negative results. The recommended algorithm for the HPV test is found in table B at Appendices 1.

Guidelines that defines all conditions of test application, has been designed/compiled from one group of expert physicians of the healthcare area, supported by the UNFPA representative office in Albania.

The training courses/workshops with the personnel offering the service in the primary healthcare system were focused in the good knowledge of the test result from the normal one to other possible pre-cancerous conditions. The knowledge given to the workshops included the follow-up guidelines as well through colposcopy for the cases of women with positive results of the test.

To decide for the patient, diagnose, was well clarified the importance of directed biopsy under colposcopy procedure. The right diagnose was followed by the recognition of the treatment options of the patient, conformed to the disease stage.

The offering of this specialized medical service needs one specialized Obstetric - Gynecologist staff as well being trained for colposcopy. This training is offered by the support of Albanian Representative Office of UNFPA, for following up the theoretical online lessons from experts of colposcopy society and for practice lessons of the knowledge in the central cabinets in University Hospitals of Obstetric - Gynecologist.

4. DISCUSSIONS

Setting up and establishment of the system for the test notification of Albanian healthy (without complaints) women at their home; the evaluation of those with problems and giving healthy solution from the specialists, requires a good coordination of all structures included in this testing.

With the support of the Ministry of Health and UNFPA, the whole chain is possible to stay and remain connected for achieving the objective of decreasing the Cervical Cancer Incidence Rates. The qualification of the personnel offering the service in the primary healthcare, as per respective medical position, was done in some piloted regions, which are thought to be the first to start the screening testing.

The expert group who compiled testing guidelines decided as appropriate the recognition of the below three screening methods of cervical cancer: i) screening method with HPV test, ii) Pap smear conventional (cytology) and, iii) visual inspections with acid acetic (VIA).

Ever since cervical cancer screening with the Papanicolaou smear has become widespread, the incidence of invasive cervical cancer has dramatically decreased. At the same time, the detection of cervical dysplasia has significantly increased. For such patients, the benefits of detecting and eradicating cervical dysplasia must be balanced against the long-term complications of treatment. The use of the term "pre-cancer" for the low-grade lesions is problematic. Some of the epithelial changes which include the morphological features of neoplasia may, in fact, be acute human papilloma virus (HPV) infections of the epithelium; majority of such lesions will not progress to high-grade intraepithelial neoplasia or to invasive cancer. In the low-grade lesions, it is difficult to predict the biological behavior of the epithelial change, but the term "pre-cancer" is still used because of the potential for such lesions to progress to high-grade intraepithelial disease and, subsequently, to invasive cancer. The optimal screening strategy should identify those cervical cancer precursors which are likely to progress to invasive disease. Also, it should avoid detecting transient HPV infection and its associated benign lesions that are not destined to become cancerous.

Diagnostic level increase, for the positive result patients, will be achieved by their follow up from Regional Hospitals, where the patients will receive

the service from the specialized Obstetric - Gynecologist physicians in colposcopy, whom are being qualified by the training sessions already started. All these follow up levels will function step by step from primary healthcare service – the first provider of the medical service up to specialized Obstetric - Gynecologist physicians of the colposcopy cabinets. Colposcopy and directed biopsy is an accepted management technique for a selected cohort of patients with abnormal screening test. When an abnormality is encountered on screening, it must be confirmed with the help of a conclusive test.

5. CONCLUSION

Ministry of Health is facing as her next challenge the organization of screening for cervical cancer and has prepared the entire supportive infrastructure to start it as soon as possible. To achieve this, Ministry of Health has the support from the Representative office of UNFPA in Albania, which has been closely, collaborate with the experts of this area of healthcare. Our vision is to reduce deaths and to give the better life free of cervical cancer to the women.

Appendices 1.

Table A. Pap Smear Test

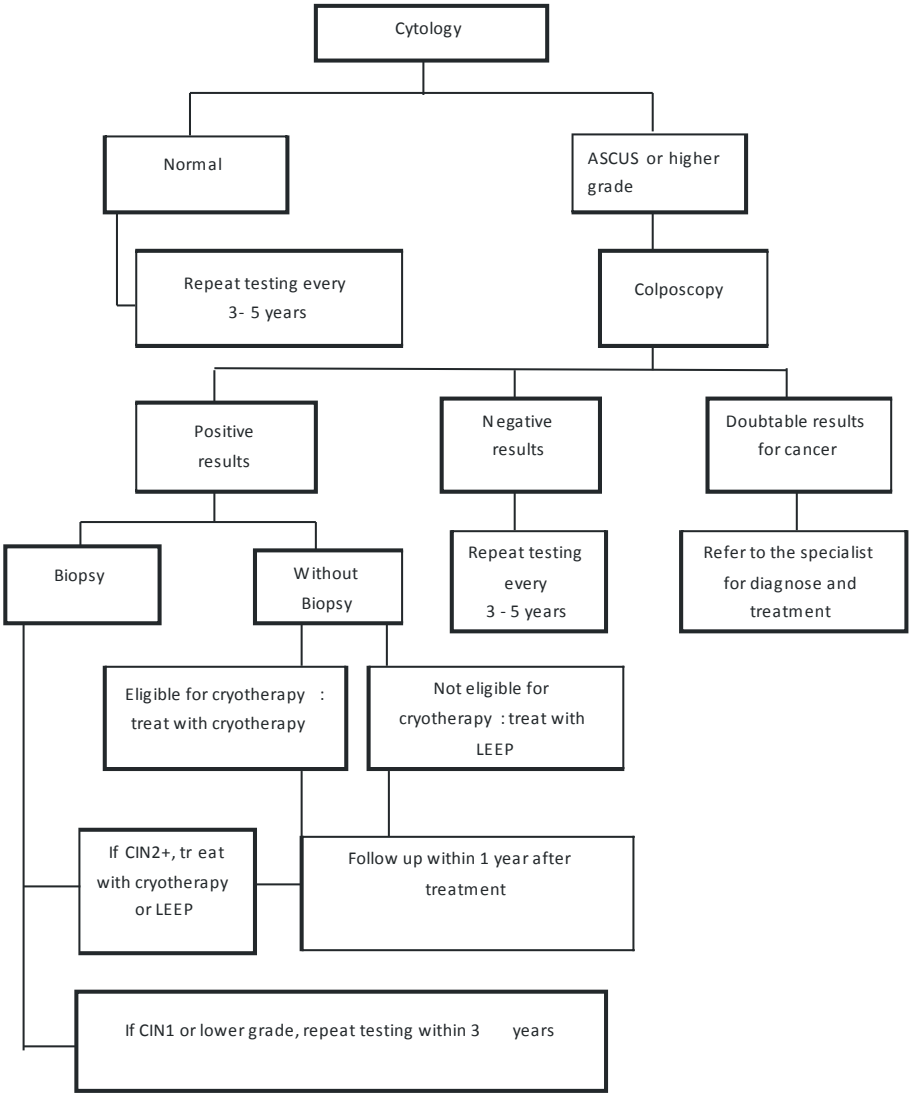
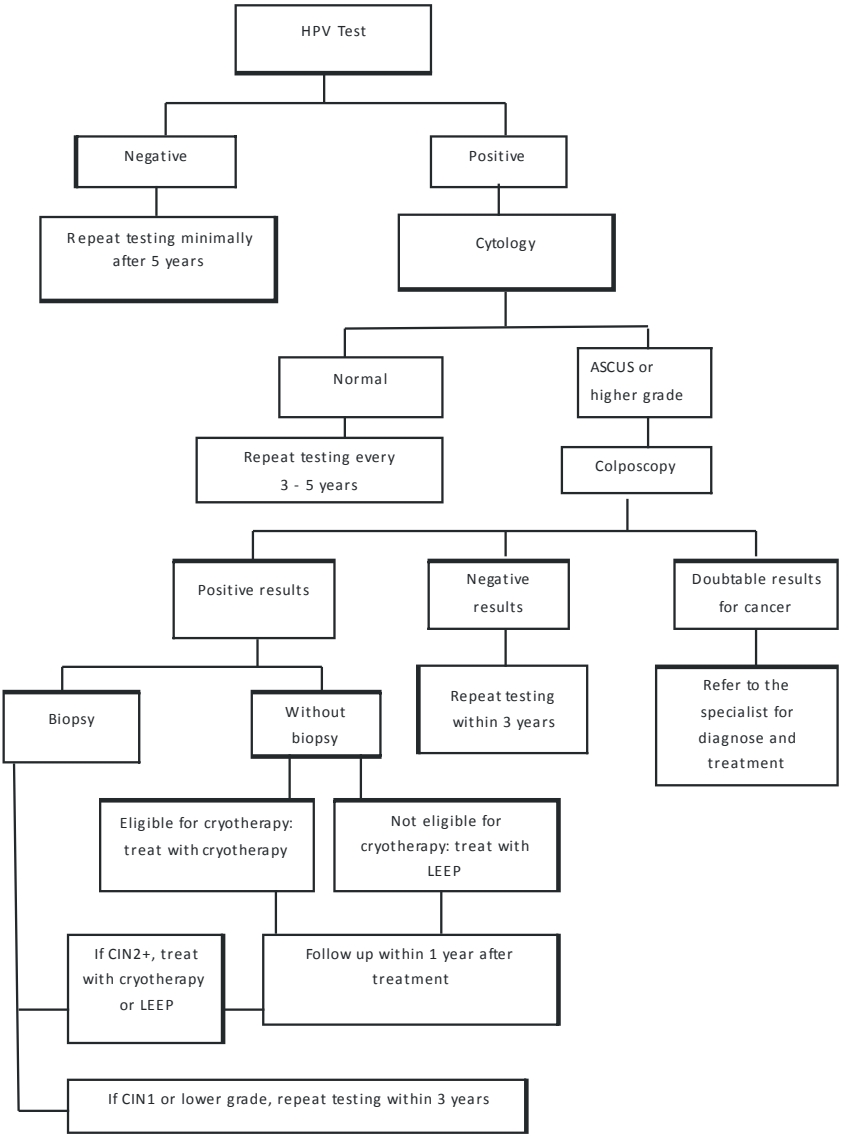


Table B. HPV TEST



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CERVICAL CANCER SCREENING: CITOLOGY VERSUS HUMAN PAPILLOMA VIRUS (HPV) TESTING IN ALBANIA

Lila SHUNDI
Institute of Public Health, Tirana, Albania
Genta NALLBANI
Catholic University, Our Lady of Good Counsel, Tirana, Albania
Brunilda VILA, Eriona ABAZAJ
Institute of Public Health, Tirana, Albania
Tritan SHEHU
Catholic University, Our Lady of Good Counsel, Tirana, Albania

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ABSTRACT

This paper briefly describes the 15 years experience in introducing HPV testing for cervical cancer screening in Albania and the efforts undertaken to generate epidemiological data on the carriage of cervical HPV DNA. The main objective was data collection and selection on HPV infections and HPV type circulating patterns, in view of identifying a proper method for the future construction of a national prevention and screening program A total of 3557 Albanian women, 17- 68 years old, were screened for cervical cancer during 2012-2016 period of time with Digene Hybrid Capture 2 HPV DNA Test (Qiagen). HPV self-sampling, significantly improved the participation in the screening. HPV cervical infection in Albania, presents a high prevalence (16.7%) despite of a relatively low incidence rate (6/100.000) of cervical cancer. HPV16 and HPV18, two high-risk HPV genotypes targeted mostly by HPV prophylactic vaccines, together accounted for 37.7 % of HPV infections in Albania. Results obtained are essential for the implementation of screening policies related to cervical cancer, and also for including HPV vaccine in the national vaccination program in Albania and, finding viable cost-effective solutions for cervical cancer control in Albania and exploration of realistic options.
Keywords: Cervical Cancer Screening, High-Risk HPV Test, Human Papilloma Virus, genotype, self-sampling, HPV-DNA

INTRODUCTION

Worldwide, there are approximately half a million cases of cervical cancer annually and 85% of cases occur in the developing world (Ferlay 1992; Ferlay 2004). Cervical cancer accounts for 10% of all female cancers, making it the second leading cause of cancer death in women. In the developed world the incidence of and mortality from cervical cancer appears to be falling, particularly in countries with systematic screening programmes (Arbyn 2009). Despite this trend in the developed countries cervical cancer remains the second most common cancer in women less than 45 years of age (Ferlay 1992; 2004).

Cervical cancer is the only cancer with a single, known cause: Human Papilloma Virus (HPV). Infection with oncogenic types of HPV is a necessary risk factor in cervical carcinogenesis (Walboomers 1999). Most high-risk HPV infections clear spontaneously but in a small proportion of women the infection persists. It is this women group who are at risk of developing high-grade cervical intraepithelial neoplasia (CIN) grades 2 or 3 and adenocarcinoma in situ, which are cancer precursors (Schiffman 2007).

The most important purposes of cervical screening are the reduction of the risk for cervical cancer through the detection of lesions that have the potential to become invasive cervical cancer and, the reduction of the risk for advanced cancer through the detection of asymptomatic or early-stage cancer (Sasieni *et al*., 2010)

In developed countries, cytology based screening programs have reduced more than 75% of incidence and mortality from cervical cancer in the last 50 years. However, there are still about 55000 annual cases of cervical cancer in the European region (Forman 2012) and, in many cases the diagnosis of cervical cancer was preceded by normal cytology screening.

Recently, HPV testing as a primary screening test has been investigated as a possible way to improve the performance of cervical screening programs (Mayrand 2007; Cuzick 2008; Franco 2009; Arbyn 2012). The results from four European randomized trials (Naucler 2007; Bulkmans2007; Kitchener 2009; Ronco 2010; Anttila 2010; Rijkaart 2012; De Kok 2012; Elfström 2014, Ronco 2014) comparing HPV-based with cytology-based cervical cancer screening, highlight the opportunity that HPV testing affords to permit enhanced detection of CIN2 and CIN3 in screened women, therefore enabling early treatment and reduction of invasive cervical cancer risk. Primary screening for HPV provides 60–70% greater protection against invasive cervical cancer than the cytology-based screening because HPV DNA test has a higher sensitivity for detection of cervical intraepithelial neoplasia, the precursor lesion of cervical cancer (Mayrand 2007; Naucler 2007; Ronco 2008; Arbyn 2012; Ronco 2012). The most important property of HPV-based

screening is the safety it brings to most women who have a negative HPV test. Therefore HPV based cervical screening programs have an increased protective effect against cervical cancer.

In Albania, as in other developing countries, cervical cancer is the third most common female cancer in women aged 15 to 44 years in Albania. Ninety five new cases are diagnosed each year on the average. An incidence of 6 new cases per 100,000 women per year and a mortality of 6 cases per 100,000 women per year has been estimated urban population results 4-5 times more affected than the rural one.

Albania is facing a complex and unclear situation in relation to screening and prevention of cervical cancer which is conditioned by the lack of a formulated national strategy and of the ensuing national programs.

In Albania, the anti-HPV vaccine is available only in private pharmacies with a price around 400 USD for three doses. There are no recommendations about the age to administer the vaccine. However, it is not mandatory for young girls. In 2010, the population coverage by the anti-HPV vaccine was still insignificant.

Recently, the guidelines regarding the early detection of cervical pre cancerous lesions and cancer have been based on WHO recommendations (WHO 2013). The new screening recommendations address age-appropriate screening strategies, including the use of cytology and high-risk HPV testing alone as a primary screening approach, follow-up, age at which to exit screening, and screening strategies for special population.

Although opportunistic cervical screening based on HPV-testing, is performed in Albania, well-prepared plans have been established for switching to organized screening in the near future.

CERVICAL CANCER SCREENING IN ALBANIA

1. Cytology

Neither a national nor a regional organized cervical screening program does exist in Albania. Only opportunistic individual screening is present. More than half of cancers are diagnosed in III/IV stage.

Based on IPH estimation, 8000-10000 Pap smears per year are performed in Albania in some gynecological-obstetrical centers and private clinics in Tirana, covering around 5% of population.

In Albania, neither a national, nor a regional organized cervical screening program does not exist. Opportunistic individual screening is present only. More than half of cancers are diagnosed in III/IV stage.

Based on IPH estimation, 8000-10000 Pap smears per year are performing in Albania in some gynecological-obstetrical centers and private clinics in Tirana, covering around 5% of population (Filipi 2014). The screening

coverage among women of reproductive age in Albania is probably the lowest in the region: only 3.2% of women 15 to 44 years old reported having ever been screened with a Pap-smear, with additional differences observed among women in urban (4.9%) and rural areas (1.8%). Also 2.7% of women 15 to 44 years old reported having had a Pap-test performed regularly every three years (4.3% of women in urban areas compared to 1.5% of women in rural areas) (Filipi 2014). Cost of health care, consults by the doctors, lack of perceived need (i.e., no symptoms), were the dominant concerns among these women. These issues kept women far away from routinely cervical screening. All these factors may be the reason of diagnosed squamous cell carcinoma (SCC) in advanced stage in Albania (Xhani 2013).

On the other hand, Pap-smear sample manipulation is not strictly regulated; it is not carried out regularly and uniformly in all territory but only in selected areas where some practitioners are trained and feel confident to do it.

The laboratory procedures are not strictly regulated as well. Laboratory technicians are simply aids of trained doctors who read in the microscope. As a norm there are not 'primary screening technicians' in Albania. This qualification does not exist. Only a limited number of doctors with specialization in pathology (virtually all in Tirana) are reading the samples as they are directly sent from hospitals. No standard evaluation or quality control exists.

- **Molecular HPV screening**

2.1 HPV testing

Molecular HPV testing was first introduced in Albania, in 2011 by the Laboratory Molecular Biology, the Institute of Public Health, Tirana. The molecular technology chosen for the detection of HPV DNA, was the Hybrid Capture 2 (HC2 - Digene Corporation, USA - Qiagen, USA), a Food and Drug Administration (FDA) approved test (since 1995) for HPV detection. Hybrid capture is a nucleic acid hybridization assay with signal amplification, meaning that the chemiluminescent or fluorescent signal is amplified to aid detection. The HC2 assay uses nucleic acid hybridization and microplate chemiluminescent detection. Specimens containing HPV DNA are hybridized with a HPV-specific RNA probe, creating a DNA:RNA hybrid molecule. The microplate well is coated with antibodies that bind DNA: RNA hybrids, thus capturing the hybrid molecules to the microplate. Alkaline phosphatase-conjugated antibodies bind the hybrid molecules, and a signal is detected on the addition of a chemiluminescent substrate. Signal amplification occurs because several alkaline phosphatase molecules are conjugated to each antibody, and multiple conjugated antibodies can bind each captured hybrid.

This assay detects infection from 13 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and, is not useful for detection of HPV specific genotypes.

Clinically regarded as the "Gold Standard" HC2, was chosen for HPV screening in Albania:

First, because it is the most widely used HPV assay in clinical laboratories and the most clinically validated diagnostic test: Over 1 million women in large randomized controlled trials (RCT´s) were tested by HC2 (Sherman 2003; Kitchener 2006, Cuzick 2008, Castle 2009, Sankaranarayanan 2009, Leinonen 2009, Ronco 2010).

Second, because of the easy laboratory implementation having an increased workflow flexibility and efficiency. HC2 can be run anywhere in the lab and there is no need for unidirectional workflow. HC2 does not need an internal control as it is antibody based. HC2 contains more controls and calibrators than any other HPV test and is the only assay to control against low-risk cross-reactivity on every plate.

Third, because HC2 distinguishes between clinically relevant and background infection. It is evaluated in numerous randomized, controlled and cohort studies demonstrating the clinical value of HPV testing in general (Snijders 2003). The Analytical Sensitivity of HC2 is 5,000 copies of HPV DNA/Assay.

Fifth, HC2 has a 99.9% Negative Predictive Value and 97% sensitivity for CIN3+.

Sixth, Taking into account that 5% of cancers contain L1 deletions (Karlsen1996, Walboomers 1999, Capone 2000, dos Santos Oliveira 2002) - HC2 is not vulnerable to deletions. Most PCR methods are based on primers targeting L1 or E1 regions of the HPV genome. These targeted segments may be eliminated when virus DNA is integrated into the human genome — a precursor for many advanced stages of the disease. Many PCR methods yield false-negative results that do not recognize a proportion of disease stages such as severe dysplasia and cervical carcinoma.

Seventh, HC2 it is validated for use with ThinPrep liquid cytology medium, Digene's cervical collection kit and, SurePath liquid cytology medium.

2.2 HPV Genotyping

During 2012-2014 period of time, a PCR-based assay for HPV genotyping was used in Albania: *digene HPV Genotyping RH Test* (Qiagen, Gaithersburg, MD), which uses the principles of reverse line blot hybridization. The digene HPV Genotyping RH test amplifies HPV DNA with GP5+/6+ consensus primers within the highly conserved L1 region of HPV genome. One membrane strip coated with multiple probes allows the simultaneous detection

of 18 individual HPV genotypes: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82.

Another commercially available test, the *digene* HPV Genotyping PS test (Qiagen, Gaithersburg, MD), based on signal amplification technology has been used since 2015. It is a reflex test intended for the individual qualitative detection of high-risk HPV types 16, 18, and 45 following a positive *digene* HC2 High-Risk HPV DNA test result. The identification of HR-HPV types 16, 18, and 45 provides additional information to aid in the clinical management of women in cervical cancer screening programs.

2.3 Sample collection

During years 2011-2012, sampling was done by clinician using *digene* DNA PAP Cervical Sampler™ Specimen Transport Medium™ (Qiagen, Gaithersburg, MD)

From 2013, self-sampling was introduced, using *Digene Female Swab Specimen Collection Kit* (Qiagen, Gaithersburg, MD).

- **RESULTS AND DISCUSSION**

A total of 3557 Albanian women, 17- 68 years old, from different regions of Albania (Tirana, Durres, Vlora, Fier, Berat, Saranda, Delvina, Erseka) were screened for cervical cancer during 2012-2016 period of time (Fig. 1).

After a two years (2011-2012) of a very low response to the invitation of IPH, for a routine HPV screening, self-sampling was introduced in the year 2013. Self-sampling, being less invasive collection method, significantly improved the participation of Albanian women in the screening from 9.6 to 11.8 folds (Fig. 2). In the meantime, self-sampling facilitated the access to cervical screening for women in low resource areas in Albania. *Digene* Cervical Sampler - the only device extensively validated for HPV testing, that was also used as device of choice in several self-sampling studies, it was used for self-sampling in Albania. Samples were analyzed with Digene Hybrid Capture 2 HPV DNA Test (Qiagen). The overall prevalence of infections with any HR-HPV type in Albania was 16.7%, close to those reported for Balkan regions and Eastern Europe (14.2%) (*Bruni L et al.2010).* Figure 3 present HPV prevalence by age in Albania, compared with different regions of the World. The age distribution of cervical HPV infection in Albania showed a bimodal curve with a first peak, as in all regions, at younger ages (<25 years), just after sexual debut and, a second peak at ages ≤ 44 years also. The second peak of HPV prevalence might be result of different factors as sexual behavior interplay, HPV type and variants, host immunity, screening practices. The prevalence of infection gradually descended to a plateau in middle-aged women.

Fig. 1: Albania's regions when HPV screening was applied.

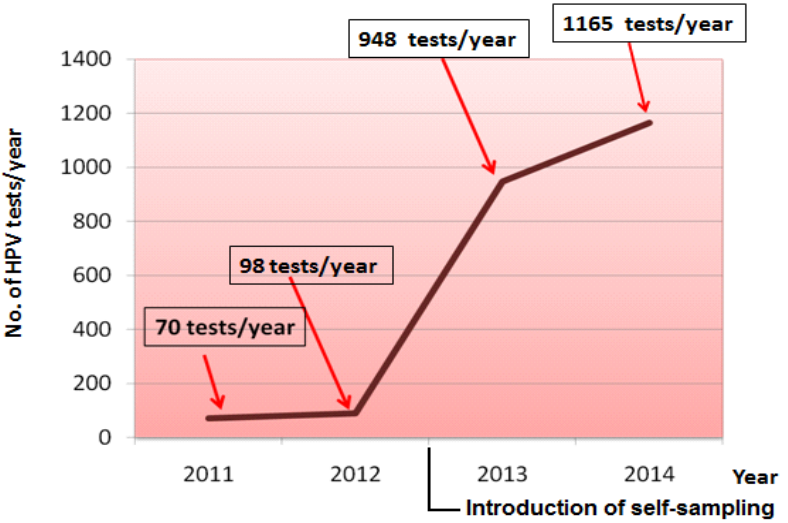

Fig. 2. Number of HPV tests performed during years 2011-2014 in Albania.

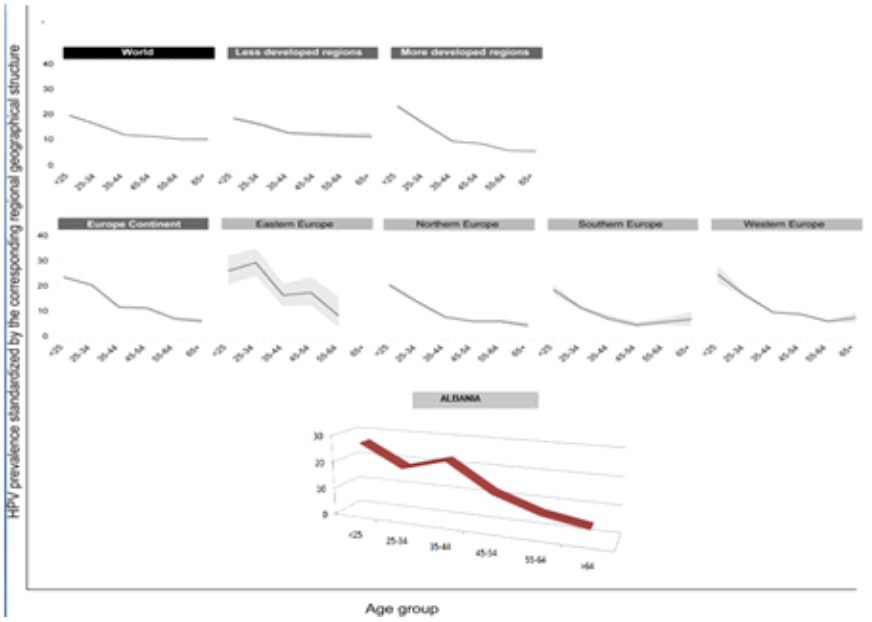

Fig. 3: Age specific prevalence in Albania compared with other regions of the World (adapted from Bruni *et al*., 2010).

Usually, the regions with high HPV prevalence are the ones with the highest cervical cancer incidences, and the regions with lower prevalence had the lowest incidences. Eastern Europe was the opposite, presenting a high HPV prevalence (21.4%) but a relatively low incidence (14.5 new cases per 100,000 women per year) (Bruni *et al.*, 2010). The same HPV cervical infection in Albania, presents a high prevalence (16.7%) despite of a relatively low incidence rate of cervical cancer (6 new cases per 100.000 women per year).

HPV cervical infection in Albania, like in Eastern Europe was the opposite, presenting a high HPV prevalence (21.4%) but a relatively low incidence (14.5 new cases per 100,000 women per year) (Bruni *et al*., 2010). presents a high prevalence (16.7%) despite of a relatively low incidence rate of cervical cancer (6 new cases per 100.000 women per year).

Screening 18-24 years old women: Baseline information on human papillomavirus (HPV) prevalence and type distribution is highly desirable to evaluate the impact of prophylactic HPV vaccines in the near future. Detailed knowledge of HPV infections and HPV type circulating patterns are essential for appropriate implementation of screening, prevention and surveillance program. To characterise HPV infection prevalence in Albania prior to implementation of prophylactic HPV vaccination and to determine local HPV genotypes specifics in order to assess the potential benefit of HPV vaccination in Albania, starting with year 2016, IPH has inviting 18-24 years old women for HPV screening. Sampling was done by self collection method, and, a total of 400 women was tested with Hybrid Capture 2 Assay, till now. Genotyping of HR-HPV positive samples is in process. Prevalence of HR-HPV infections in 18-24 years old women in Albania resulted 26.75%.

Small-scale pilot program: On July 2016, IPH started a small-scale pilot program at three primary health centers belonging of two regions of Albania: Tirana and Fieri. The main objective was screening for HPV infection of 3000 women 30-65 years old. Sampling was done by self collection. Samples were analysed with Digene Hybrid Capture 2 HPV DNA Test (Qiagen). Till now 469 women were screened in Tirana with a positivity of 8% for HPV infections and 400 women were screened in Libofsha with positivity for HPV infections 6%.

Distribution of high-risk HPV types in Albania. There is considerable heterogeneity in the risk and distribution of HR-HPV types across regions worldwide, which may lead to differing cervical cancer incidences and mortalities among the countries. Thus understanding the type-specific distribution of HR-HPV and its attribution to different grades of cervical lesions will direct the implementation of successful programs for cervical

cancer prevention and management. The design of adequate strategies for surveillance of vaccinated as well as non-vaccinated women in Albania is important to obtain detailed data of the circulating HPV genotype patterns. We have estimated the pre-vaccination prevalence of cervical infections with 13 HR-HPV types. This study is the first to establish the distribution of HPV genotypes in a relatively large population in Albania. A total of 12 different HPV genotypes were detected. The most common HPV types found among Albanian women were the HPV oncogenic types: 16, 18, 31, 45, 51, 52, 53, 56, 58, 59, 66, 68.

Similar to other European countries, cervical infection with HPV 16, the HPV type with the strongest oncogenic potential, was most common among Albanian women: 30.5% of HPV infections were estimated to be produced by HPV 16, similar to global HPV burden. HPV 18 type is the second most prevalent type (7.2 %). HPV 31 type – especially frequent in Europe, has a 6.6% prevalence in Albania. HPV 56, 45,51 shared similar prevalence. HPV type circulation in Albania is far more complex because of association of general features (HPV16 most prevalent type worldwide) with specific features (HPV53 with 3% prevalence, HPV18 relatively rare in Southern Europe).

The baseline pre-vaccination distribution of HPV genotypes established in this study will be very helpful for monitoring potential HPV genotype replacement under the selective pressure of HPV vaccine.

Our results provide data for monitoring the impact of Albanian HPV vaccination program and development of future cervical cancer screening strategies in cohorts eligible for free HPV vaccination.

Our data could be used for health economics, to determine cost-effectiveness analysis of a future cervical screening and HPV vaccination program implementation in Albania

A major challenge in Albania will be raising effective diagnostic and treatment capacity to the level of being accessible to the entire population, not just the women attending screening programs. Developing and testing a sustainable model for population-based cervical cancer screening could focus attention on the most cost-effective steps that could be undertaken to improve diagnostic and treatment protocols.

Further progress in Albania to develop an effective and sustainable approach to early detection of cervical cancer will require additional investment of resources and technical assistance through international collaboration, especially through European networks.

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PREMALIGNANT DISEASES OF CERVIX UTERI

Nikita MANOKU and Enriketa KROI
University Hospital of Obstetrics-Gynecology, Queen Geraldinë, Tirana, Albania

ABSTRACT

Cervical cancer is one of the most common gynecological neoplasms, ranked first in the developing countries and second in the worldwide range after breast cancer. It is estimated that it forms about 6% of tumoral pathologies in women. This disease is most commonly found in women aged 35-45 y/o in the invasive form and 31-33 y/o in the intraepithelial form. Invasive cancer of the cervix is considered a preventable disease because it has a long preinvasivestate, cervical cytology screening programs and effective local treatment. All these have led to a mortality decrease at about 70% in the last 50 years. CIN (cervical intraepithelial neoplasia) known as the preinvasive state of cervical cancer leads to malignant/invasive cervical cancer in about 15-70% of the cases in approximately 7 years.
Keywords: CVC, women aged 35-45y/o in the invasive form, 31-33 y/o in the intraepithelial form

ETIOLOGY

The etiology of the illness relates to : i) HPV, Human Papilloma Virus .Infection with HPV is the primary cause of cervical cancer .Only certain types of HPV count for 90% of high grade intraepithelial lesions and cancer. There are several types of HPV ,16,18,31,33,35.Type 16 is the most common HPV found in invasive cancers and CIN 2,3(47%). Type 18 is found in 23% of women with invasive cancers and 5% of woman with CIN 2,3. Most types of HPV lead to condilloma forming in different parts of the body and have usually a benign course. But type 16 and 18 are considered 'High Risk'. HPV-DNA has been detected in most women with cervical neoplasia(50-80%), ii) HIV. HIV patients have a high incidence of CIN and invasive cancer,as a result of immunodeficiency and HPV infections, iii) HSV2 and EBV have been found in several cases of invasive cancers but there is no scientific proof

yet, iv) sexual behavior , multiple partners not protected sexual intercourse leads to HPV infections which might lead to cervical cancer, v) oral contraceptives. Some studies have shown that oral contraceptives can cause cervical cells to transform into neoplastic cells. This may also be as a result of periodic screening tests that this group undergoes, which may lead to early diagnosis and, vi) smoking. DNA changes related to smoking have been found in cervical mucus.

PHYSIOPATHOLOGY OF PREINVASIVE DISEASE

Transformation zone

Exocervix, or the vaginal portion of the cervix is covered in squamous epithelium (stratified). Cervical canal (endocervix) is covered in columnar epithelium. The junction between these two different epithelia (squamocolumnar junction) is located in different parts of the cervix in response to puberty, pregnancy, menopause, hormonal stimulation. In neonates and children it is located at the external os of the cervix. In puberty and pregnancy it is located in the vaginal portion of the cervix. At menarche the production of estrogen causes the vaginal epithelium to fill with glycogen. Lactobacilli act on the glycogen to lower the PH, stimulating subcolumnar reserve cells to undergo metaplasia. Metaplasia advances from the original squamocolumnar junction inward toward the external os and over the columnar villi. This process establishes an area called the active SCJ.

Dysplasia

Exogene and oncogene factors are introduced during sexual intercourse. Several agents including sperm, seminal fluid histones, trichomonas, chlamydia and HSV have been studied. It is now known that HPV plays an important role in the development of cervical dysplasia (CIN). Dysplastic cells have larger nuclei and a higher proliferative rate than squamous cells.

Cervical dysplasia can be mild (CIN 1), moderate (CIN2), severe (CIN3)

CYTOLOGICAL SCREENING

Papanicolau test (pap test) has been successful in reducin the incidence of cervical cancer by 79% and its mortality by 70% since 1950. It was first introduced by Georges Nicholas Papanicolau in 940.

Papanicolau classification

Class I absence of atypical or abnormal cells (negative result)

Class II atypical cytology but no evidence of malignancy (negative result)

Class III cytology suggestive but not conclusive for malignancy (non-conclusive result)

Class IV cytology strongly suggestive of malignancy (positive result)

Class V cytology conclusive for malignancy (positive result)

Bethesda classification system

General categorization

Negative for intraepithelial lesion or malignancy

Epithelial cell abnormality (squamous/glandular)

Other (endometrial cells)

Epithelial cell abnormalities

Squamous cells

Atypical squamous cells (ASC)

Atypical squamous cells of undetermined significance (ASC-US)

Atypical squamous cells, cannot exclude HSIL (ASC-H)

LSIL (low grade squamous intraepithelial lesion) CIN 1, mild dysplasia, HPV infection

HSIL (high grade squamous intraepithelial lesion) moderate/severe dysplasia, CIN 2 and 3, CIS

Squamous cell carcinoma

Glandular cell abnormalities

Atypical (endocervical, endometrial)

Atypical favor neoplastic cells

Adenocarcinoma in situ

COLPOSCOPY FINDINGS

Colposcopy was first used in Germany in 1925 by Hans Hinselmann. A colposcope is basically a microscope with a 5-20x magnifying lens. It is used to see dysplastic changes in the epithelia of the exocervix.

Epithelium that turns white after the application of acetic acid 3-5% is called acetowhite epithelium. The application of acetic acid coagulates the proteins of the nucleus and cytoplasm and makes the cells appear white and opaque. Mature epithelial cells have small nuclei and contain glycogen.These areas appear pink during colposcopy. Dysplastic/immature cells contain large nuclei with large amounts of chromatin (proteins),and these are the cells that appear white.

Punctation dilated capillaries terminating on the surface appear as a collection of dots and thus are referred as punctuation. The puctate vessels are formed as the metaplastic epithelium migrates over columnar villi. When these vessels occur in a well demarcated area of acetowhite epithelium they indicate abnormal epithelium, most often CIN.

Mosaic terminal capillaries surrounding roughly circular or polygonal shaped blocks of acetowhite epithelium crowded together, are called mosaic because their appearance is similar to mosaic tiles. Mosaicism tends to be associated with higher grade lesions such as CIN 2 and 3.

Atypical vascular patterns are a characteristic of invasive cancer

TREATMENT

Pap test class II/ negative cervical biopsy but with squamous metaplasia requires Pap test every 6 months and colposcopy every year.

This group has a 9-33% risk to develop dysplasia in a 2 year period.

Cervical dysplasia with negative endocervical findings requires local treatment.

Cervical dysplasia /positive findings in endocervical curettage requires cervical conisation.

Micro invasive neoplasia requires conisation.

Pap test class III-V with negative findings in colposcopy or endocervical curettage, requires diagnostic conisation.

Treatment methods

Without treatment it is possible the progression of CIN 2, 3 to invasive cancer in 33% of the cases in a 2-15 years period.

Treatment modalities are as following: i) Laser ablation, ii) Cryotherapy } Destruction (ablation), iii) LLETZ/ LEEP, iv) Laser cone, v) knife cone biopsy } excision and, vi) Hysterectomy

Local therapy consists in complete destruction/excision of the dysplastic tissue minimally in 5-7mm thickness. Positive results of these treatments are seen in 85-95% of the cases.

In case of recidivation (which might occur in 5-15% of the cases, especially the first two years following the treatment (85%) its necessary to repeat : i) pap test and colposcopy after 3 months, ii) Pap test every 6 months, iii) Colposcopy, annually the first two years and, iv) hysterectomy is the last treatment in cases of unresponsive local treatment.

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CYTOMOL EXPERIENCE IN LIQUID BASED CYTOLOGY, COMPUTER ASSISTANCE, HPV AND P16/KI-67 BIOMARKER TESTING IN ROUTINE. (COMPARED TO CONVENTIONAL CYTOLOGY AND IN CORRELATION WITH HISTOLOGY)

Arjola XHAJA and Hans IKENBERG
MVZ Cytomol, Frankfurt am Main, Federal Republic of Germany

ABSTRACT

While there is mounting evidence that the widespread application of the cytological method has had a positive impact on the decreased incidence and death rates from cancer of the uterine cervix, a comparable decrease in precursor lesions has not been observed in most laboratories, yet. After decades of experience, applying the technique at the highest possible levels of sensitivity and specificity showed that the conventional Pap test suffered from a low sensitivity. It seems that the PAP-Test has reached the limits. ~50% of cervical cancer patients had < 5 years "normal" PAP smears. Sensitivity of a single cytology test for the detection of cervical intraepithelial neoplasia of grade 2or higher (CIN 2+ or high-grade CIN) is unsatisfactorily low. This problem prompted the development of new tools to circumvent it. First liquid-based cytology (LBC) and computer-assisted reading occurred, followed by HPV testing and other molecular markers. Of these new tools, HPV tests have rapidly achieved remarkable success in cervical cancer screening due to their high sensitivity and negative predictive value. Similarly, the LBC sample collection media have proven to be powerful tool that enable the application of wide range of techniques to investigate molecular markers. Detection of over-expression of p16INK4a, a biomarker of transforming HPV infections and pre-cancerous cervical lesions, has been shown to be an efficient tool in managing patients with atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous lesion (LSIL) cytology results, and for triaging HPV positive women and in some cases cytological positive HPV negative women. Recently the sensitivity of high-risk HPV testing for invasive cervical cancer (ICC) has been questioned. Meanwhile p16/Ki-67 (Cintec Plus®, Roche Ventana, Mannheim, Germany) is the best validated biomarker in cervical cancer screening. We therefore correlated HPV and p16/Ki-67 status of patients with CIN 2+ tested before biopsy and/or therapy (maximally six months earlier) with the respective histology results

1. INTRODUCTION

There is clear evidence that the widespread application of cytology has led to decreased incidence and death rates from cervical cancer. A comparable decrease in precursor lesions has not been observed in most laboratories. Applying the technique at the highest possible levels of sensitivity and specificity is obvious that the conventional Pap test has a relatively low sensitivity. The sensitivity of a single cytology test for the detection of cervical intraepithelial neoplasia of grade 2 or higher (CIN 2+) is ~70-50%.

Meanwhile several newer technologies are available to overcome these limits. In morphology liquid-based cytology (LBC) and computer-assisted reading (CAS) have set new standards. The LBC sample collection media allows the parallel or consecutive application of a wide range of techniques without a new examination of the woman. HPV tests have rapidly achieved remarkable success in cervical cancer screening due to their high sensitivity and high negative predictive value.

Finally, detection of over-expression of p16INK4a, a biomarker of transforming HPV infections and pre-cancerous cervical lesions, has been shown to be an efficient tool in managing patients with atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous lesion (LSIL) cytology results, and for triaging cytologically negative and HPV positive women and in rarer cases cytologically positive and HPV negative women.

Major HPV screening studies revealed an extremely high sensitivity of high-risk HPV testing for CIN 2+/3+.[1] In addition they proved a significantly lower rate of invasive carcinomas in the following years compared to cytology primary screening. However, some recent studies found a lower HPV prevalence in cervical cancer and its precursors[2] and a lower rate of sensitivity especially if the HPV test had been performed more than 6 months before histology.[3] One major evaluation also detected a low rate of HPV positivity preceding the diagnosis of invasive cervical cancer.[4] Meanwhile p16/Ki-67 (Cintec Plus®, Roche Ventana, Mannheim, Germany) is the best validated biomarker in cervical cancer screening.

2. OBJECTIVES

No. I. We correlated HPV and p16/Ki-67 status of patients with histologically confirmed CIN2+ tested before biopsy and/ or therapy (maximally six months early) with the respective histology results.

No. II. We also compared the finding rates with conventional cytology and LBC with CAS. Among > 400.000 routine privately insured patients from 2007 to 2015. (Cytomol data) **Fig.7**.

3. METHODS

No. I. All cases of a German commercial laboratory where histologic exams were performed after diagnostic or therapeutic procedures (colposcopically directed biopsy, conization or hysterectomy) executed in 2012 and 2013 and results of DNA tests for HPV high-risk types and/or p16/Ki-67 analyses were available were evaluated. HPV Tests were made out of cervical smears taken in proprietary tubes or in Thinprep vials (Hologic, Wiesbaden, Germany), p16/Ki-67 analyses out of Thinprep vials according to the manufacturers instructions. 80% of the HPV tests were performed with the HC2® (Qiagen, Hilden, Germany) and 20% with the cobas® test (Roche Diagnostics, Mannheim, Germany). The reason for that was a shift of the lab from HC2 to Cobas in the middle of 2013. The cases reported here have had HPV testing and/or p16/Ki-67 analyses to nearly 100% following cytological abnormality according to the Munich cytology nomenclature II. Data were drawn from the lab`s computer soft-system (zytofix®, nexus, Frankfurt, Germany). Cases were counted as one if the patient had more than one intervention in 2012/2013 resulting in a histology report. In that case the highest grade histology result was used. Routine histology was performed in external institutes for surgical pathology. Results were retrieved from external Ob/Gyn practices which had sent cytology and HPV specimens to Cytomol or directly from the pathology institutes. Because it took time to obtain as many histology results as possible, we choose to analyze the cases of 2012/13 where this goal had been achieved to an obtainable maximum. To clear discrepancies and to eliminate duplicates all extracted data were double-checked.

No. II. Since 2007 at Cytomol all LBC specimens have been processed with the computer-assisted Then Prep-Imaging-System (TIS). In Germany LBC is reserved to privately insured and self-paying patients while public healthcare only reimburses CC. To avoid bias we limited this analysis to privately insured patients. Cytologic diagnoses originally reported in the Munich Nomenclature II (MN; with the use of the unofficial Pap IIw category) until 30.06.2014, from then in the MNIII (which is still the reporting standard in Germany) were translated to TBS (The Bethesda System).

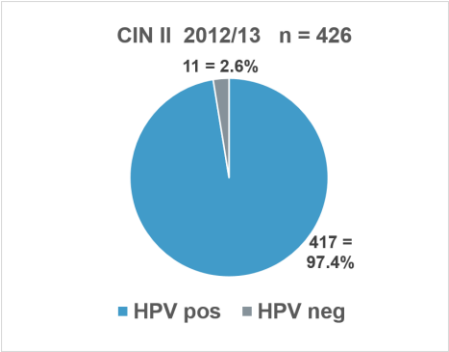
4. RESULTS

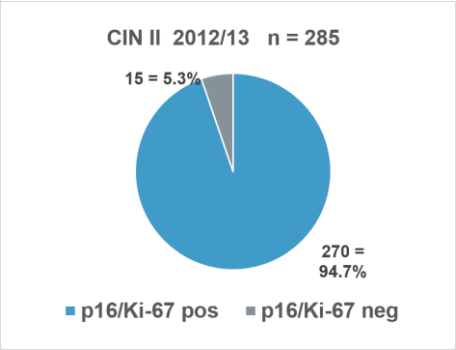
No. I. 2171 histology reports after diagnostic or therapeutic procedures executed in 2012/13 were retrieved. In 1713 of these cases (78.9%) a result of an HPV test was available (83.8 % of 511 CIN 2, 79.9% of 1549 CIN 3 and 43.2% of 111 invasive cervical cancers (ICC) **(Fig 1)**. 97.4% (417) of 428 CIN 2, 98.1% (1214) of 1237 CIN 3 and 89.6% (43) of 48 ICC tested were HPV positive, while 2.6% (11) of CIN 2, 1.8% (23) of CIN 3 and 10.4% (5) of ICC were HPV negative. Altogether 97.7% (1674) of 1713 CIN 2+ were HPV positive and 2.3% (39) HPV negative **(Figs.2-6)** Overall 2.2% of HC2 and 3.4% of cobas tests were HPV negative.

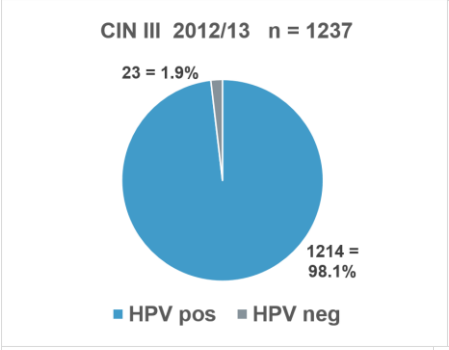
In 1252 cases (57.7%) a p16/Ki-67 result was available (55.8% of 511 CIN 2, 60.7% of 1549 CIN 3 and 24.0% of 111 invasive cervical cancers (ICC) (Fig.1) 94.7% (270) of 285 CIN 2, 99.8% (939) of 941 CIN 3 and 100% of 26 ICC were p16/Ki-67 positive, while 5.3% (15) of CIN 2, 0.2% (2) of CIN 3 and 0% of ICC were p16/Ki-67 negative. Altogether 98.6% (1235) of 1252 CIN 2+ were p16/Ki-67-positive and 1.4% (17) negative **(Figs. 2-6).** Among the 27 HPV negative CIN 2+ cases where p16/Ki-67 tests were performed 25(93%) were positive. The percentage of HPV positive and negatives did not differ significantly between CIN 2 and 3. 43 of 48 (89.6%) invasive cervical carcinomas where an HPV result was available were HPV positive while five (10.4%) were HPV negative. For ICC (26) p16/Ki-67 tests were 100% positive.

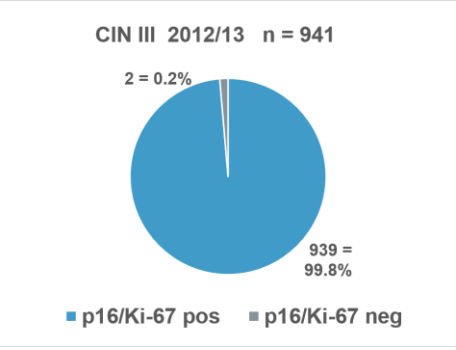
HC2 80% COBAS 20%	CIN2=511	CIN 3=1549	ICC=111	CIN2+= 2171
HPV Test	428/511 **83.75%**	1237/1549 **79.85%**	48/111 **43.24%**	1713/2171 **78.9%**
p16/Ki-67 Test	285/511 **55.77%**	941/1549 **60.74%**	26/111 **23.42%**	1252/2171 **57.66%**

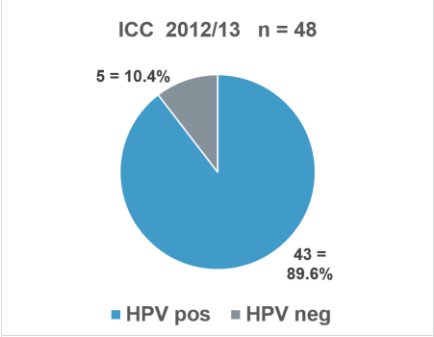
Fig. 1: HPV test and p16/Ki-67results among CIN 2, CIN3 and invasive cervical cancer**.**

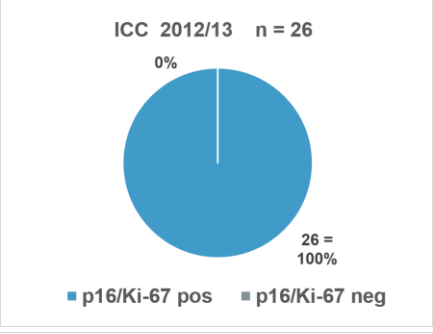

CIN II 2012/13 n = 426
11 = 2.6%
417 = 97.4%
HPV pos
HPV neg

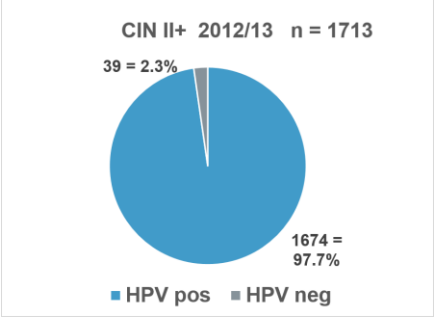

CIN II 2012/13 n = 285
15 = 5.3%
270 = 94.7%
p16/Ki-67 pos
p16/Ki-67 neg

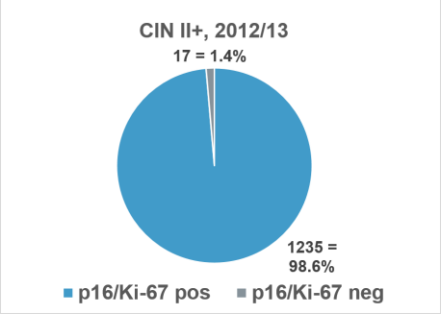

CIN III 2012/13 n = 1237
23 = 1.9%
1214 = 98.1%
HPV pos
HPV neg


CIN III 2012/13 n = 941
2 = 0.2%
939 = 99.8%
p16/Ki-67 pos
p16/Ki-67 neg


ICC 2012/13 n = 48
5 = 10.4%
43 = 89.6%
HPV pos
HPV neg


ICC 2012/13 n = 26
0%
26 = 100%
p16/Ki-67 pos
p16/Ki-67 neg


CIN II+ 2012/13 n = 1713
39 = 2.3%
1674 = 97.7%
HPV pos
HPV neg


CIN II+, 2012/13
17 = 1.4%
1235 = 98.6%
p16/Ki-67 pos
p16/Ki-67 neg

HC2 80% COBAS 20%	CIN2	CIN 3	ICC	CIN2+
HPV positiv	417/428 **97.42%**	1214/1237 **98.14%**	43/48 **89.58%**	1674/1713 **97.72%**
p16/Ki-67 positive	270/285 **94.7%**	939/941 **99.78%**	26/26 **100%**	1235/1252 **98.64%**

Figs. 2-6: Percentage of HPV positives and negatives among CIN 2, CIN 3, invasive cervical cancer and CIN 2+.

No. II: TIS had a rate of LSIL (low grade intraepithelial lesions; MN III: PAP IIID1) of 2.04% compared to 0.51% for CC, an increase of 300%. HSIL (MN III: PAP IIID2+ PAP IVa/b) was found in 1.14% with TIS vs 0.34% with CC (+225%).The ASC-US rate (MN III: PAP II-p + III) was 2.60% with TIS and 1.31% with CC, an increase of 101% which is much lower than the rise in LSIL and HSIL. It is therefore suggestive that the higher sensitivity of TIS was achieved without lowering specificity. All these results remained stable over the 9 years analyzed. **(Fig. 8-9)**

	Private cases 2007 - 2015	
	n	%
All Pap I - V	412.585	100.0%
Thin-Prep + Imager	286.161	69.4%
Conventional Cytology	126.424	30.6%

No.7. Privately insured patients from Cytomol, screened with CC and Thinprep + Imager (2007/2015)

		ThinPrep + Imager		Conventional Cytology	
TBS	Pap Group (MN II/III)	n	%	n	%
WNL	I/II / I	269.614	94,22%	123.682	97,83%
ASC-US	IIW / II pg	7.355	2,57%	1.607	1,27%
ASC-H / AGC	III / III pg	88	0,03%	47	0,04%
LSIL	III D / III D1	5.829	**2,04%**	641	**0,51%**
HSIL	III D / III D2	2.720	**0,95%**	344	**0,27%**
LSIL + most HSIL	**III D All**	8.549	2,99%	985	0,78%
HSIL	IV a/b / IV a/b pg	544	**0,19%**	89	**0,07%**
Cancer	V / V pg	11	0,004%	14	0,01%
≥LSIL + most HSIL	**≥III D**	9.104	**3,18%**	1.088	**0,86%**

No.8. Thinprep+ Imager versus conventional cytology (Cytomol Data from 2007-2015)

Year	LSIL + HSIL ~ Pap ≥IIID	% amongTP	% among CC	Relative finding rate
2007		2.68%	0.57%	4.70
2008		2.43%	0.51%	4.76
2009		2.20%	0.57%	3.86
2010		2.59%	0.65%	3.98
2011		3.75%	0.91%	4.12
2012		3.87%	1.17%	3.31
2013		4.06%	1.22%	3.32
2014		3.28%	0.88%	3.73
2015		2.97%	0.81%	3.67

No.9. Results in different years

5. DISCUSSION AND CONCLUSIONS

In routine use of a commercial lab TIS provided improved screening quality and higher productivity at the cost of higher technical expenditure. Also high-risk HPV testing in routine has a very high sensitivity for CIN 2, 3 and ICC. Most of the rare HPV-negative cases were positive for the biomarker p16/Ki-67. Liquid based cytology (Thin Prep) with computer assistance, HPV test and the prognostic marker p16/Ki-67 in case of borderline and low-grade abnormalities used as a triage deliver only patients with a high probability of a relevant lesion to invasive procedures and improve the sensitivity of the conventional cytology with the same specificity. Improved cytology and HPV testing with biomarkers complement each other and may achieve a very high sensitivity for high grade cervical dysplasia (up to 98-99%).

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[1]**Ronco G, Dillner J, Elfström KM et al. 2014.** Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet; 383: 524-32. doi: 10.1016/S0140-6736(13)62218-7.

REPORTING PAP SMEARS-AN EXPERIENCE OF TWO COUNTRIES

Blerina ÇELA, Majlinda IKONOMI, Gentiana CEKODHIMA
National University Hospital Center, Mother Theresa, Tirana, Albania
Sonela SHUXHO
Regional State Hospital, Vlora, Albania
Eriselda BASHARI
National University Hospital Center, Mother Theresa, Tirana, Albania

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The Pap smear, also called a Pap test, is named after Georgios Papanikolaou, the doctor who determined that this was a useful way to detect signs of cervical cancer. Among other screening procedures, it tests for the presence of precancerous or cancerous cells on the terine cervix.

It is the most cost effective method of prevention and detection of cervical cancer. In abnormal Pap smear, colposcopy, endocervical curettage and biopsy are recommended and the Gold standard method in detecting the cervical lesions is biopsy.

There are two methods for preparing a specimen for cervical cytology: the conventional Pap smear and the liquid-based, thin layer preparation. For both methods, cells are obtained from the external surface of the cervix (ectocervix) and the cervical canal (endocervix) to evaluate the transformation zone (squamocolumnar junction), which is the area at greatest risk for neoplasia.

The age span that most women should get regular Pap smears is starting from age 21 (or earlier in correlation with the sexual activity) and up to the age of 65, if the data show a history of normal Pap test results. For the women over 30 with three normal Pap tests in a row, this test can be performed every five years if it is combined with a human papillomavirus (HPV) screening.

It is critically important to recognize that the women who are at highest risk for abnormal Pap smear testing are those who are not getting regular Pap testing.

In order to improve the cervical cancer screening, as it is one of the most common causes of cancer worldwide and also one of the most preventable

and treatable cancers, several campains are developed to scree and educate the population, especially in certain population groups as older women, the uninsured, rural areas, etc.

One of the important parts of achiving these goals is also standardizing the way of reporting the two main categories of papsmear results, the normal and abnormal categories. In order to improve this, in Albania, in cooperation with the Albanian Academy of Science, during the International Science Conference "Cervical cancer: Prevention, diagnosis and treatment", it was held also a seminar with pathologists and cytologist all over Albania, and with the special participation of specialists from Germany.

During this seminar were adressed issues related to the challanges we face in interpreting the papsmears in order to facilitate the assignement into the low-risk category or high risk category and accordingly minimize the cases of ASCUS or AGUS that place the patient, clinician and the pathologist in an uncertain position regarding what should be done for the patient.

During the discussion of the several cases brought from Albania with the corresponding biopsy samples when possible, and also the cases brought from Germany, several problems were highlighted:

Starting with the relevant accompaning information, in order to gain the greatest amount of useful information from a Pap smear, we need clinical information about the patient regarding the age, date of last menstrual period, pregnancy status, and the history of previous abnormal Pap smears.

Air-drying artifacts are the Pap smear enemy number one. If the cell dryes before it is spray-fixed, it enlarges causing this way the lost of the nuclear details. If the pathologist cannot determine if a dried cell is atypical or not, he may tend to class the smear as an ASCUS. What needs to be done in order to avoid this is fix the smear immediately with the appropriate spray.

Blood, mucus, and pus serve to obscure and distort the epithelial cells, making it difficult to determine if they are atypical. If you do the conventional papsmear you should before collecting the specimen, remove the excess mucus in order to reduce the amount of blood on the smear, use the spatula first and then the brush (because the brush is more likely to induce bleeding). One final tip is to not performe Pap smears while the patient is menstruating, as when a woman is menstruating she sheds cells from the lining of her uterus, the endometrial cells. If these cells are seen on the Pap smear the report may note that these cells are present and can be mistaken for malignant (cancerous) cells.

Since there exist different laboratories that perform paptest exams and interpretation, but not all are part of hospital structures equiped with the propriate instruments for biopsy sampling, there exists also a handicap regarding the correlation between papsmears and biopsies in the cases of abnormal categories that require biopsy. The pathologist that examines the

papsmear is not always the one who examines also the biopsy and this may be subject of over or underdiagnosis. The abnormal Pap and follow-up biopsy should be examined simultaneously by the same pathologist because cytopathological correlation is the cornerstone of cervical pathology. This is important in order to determine the significance of the abnormal Papsmear, to determine also if the biopsy was sampled in the right area of the cervix, since studies have shown that a very significant proportion of "ASCUS" Papsmears are found to be high-grade lesions.

In the laboratories in Albania, the most used technique is the conventional papsmear, as the most costeffective method of screening. But what about ThinPrep? ThinPrep is the first of the liquid-based technologies that prepare technically superior preparations without having the problems of drying and other artifacts. However, this method is slightly more sensitive for high grade and low grade squamous epithelial lesions and there is no evidence of superiority regarding the diagnosis of adenocarcinomas and other glandular lesions.

Besides this, the experience has shown that there also limitations to ThinPreps in comparison to the conventional pap smears. Low grade squamous lesions (LSIL) are easier to recognize on ThinPrep than on conventional Pap smears, but high-grade lesions (HSIL), in conventional papsmears can be readily recognized as inconspicuous, small, round cells of HSIL that are typically grouped together. In ThinPreps, the cells suspension is more homogeneous, and HGSIL cells are located apart from each other and are much more difficult to spot. Also, in ThinPreps it is difficult to distinguish adenocarcinoma cells from normal endometrial cells.

In the laboratories abroad besides ThinPreps, in the cases of full coverage from the insurance policies, which is another problem regarding the implementation of this method because of its cost in comparison to conventional papsmears, there are also other exams which are performed on the material sampled and that has resulted abnormal. Immunohistochemical exams are another important topic to mention in this discussion. The experience showed by the specialists from Germany, has revealed new insights on the importance of this method in the diagnosis and follow-up of abnormal category. Here in Albania, the financial aspect of these exams limits their use in the everyday practice.

The recent projects in our country are designed to include also the definition of HPV status in the albanian population, since there are no relevant data for this important factor in the cause of cervical cancer. The principal risk factor is infection with the genital wart virus, also called the human papillomavirus (HPV), although most women with HPV infection do not get cervical cancer. Almost all cervical cancers are related to HPV infection.

Some women are more likely to have abnormal Pap smears than other women.

HPV is a sexually transmitted virus that may be spread from one person to another even when the genital sores are not visible. Many sexually active people are carriers of HPV, very often without even knowing they are carriers. It is estimated that up to 60% of sexually active women harbor this virus on their cervix or in their vaginal area. It is not unusual for a woman to be unaware that she has HPV - only to find out that her Pap smear shows evidence of HPV.

HPV is not curable, although the cellular damage it causes is generally treatable and vaccines against the most commonly found HPV types are available.

The main use of HPV testing in screening for cervical cancer is to determine the treatment and follow-up recommendations for women with Pap smears interpreted as atypical squamous cells of undetermined significance (ASC-US).

The final Pap smear diagnosis is based on the patient's history, the sample adequacy and the presence or absence of cellular abnormalities. Once the final diagnosis is made, the follow-up recommendations orient on the next steps to be taken.

Taking all these in consideration, it is important to develope a close working relationship between gynecologists and pathologists, as the gynecologist has all the clinical information and the pathologist has all the microscopic morphologic information, which make possible to complete the clinical picture.

Conference Document
RECOMMENDATION FOR HEALTH POLICIES

Tirana, 4th of February, 2017

Scientists, experts and professionals from various health institutions in Albania and other countries of the region, gathered in the conference **"Cervical-cancer: prevention, diagnosis and treatment"***, organized in the Academy of Sciences of Albania,*

Underline:

- **Cervical cancer is the second most frequent cancer, behind only breast cancer, in women of reproductive age (15-49 years old) in Albania. Every year around 40 women die in a relatively young age from this cancer.**
- **Deaths from cervical cancer are considered preventable. With existing knowledge and technology there should not be deaths caused by this cancer. Screening programs for cervical cancer are considered very cost effective and would provide tangible benefits for all society.**
- **Time trends of cervical cancer occurrence in Albania are not showing any sign of decrease. The organization and the support for existing services are insufficient, ineffective and inappropriate, for preventing cervical cancer in population. In Albania, there are provided only opportunistic screening services, with a very low population coverage, without guaranteeing elements of ethics and poor quality control.**

Research/academic/health Institutions in Albania have recently developed a large body of information, sufficient for providing the detailed analyses of capacities, gaps and needs at different levels of health system, to set up a national organized screening program. Based on that information, Conference strongly recommends:

- **Development of organized screening for cervical cancer in Albania. There is not yet a national programme for HPV vaccination in the country. Nonetheless, even in the prospect of the provision of HPV immunisation, the screening program remains indispensable; if vaccine**

would be available today, its effects in the women's health would be relevant in 30 or even 40 years.

- **In order for a screening programme to be effective and with minimal risks for population health, it should be organized and assure a large screening coverage, while assuring in the same time quality control in all levels of the health system. A screening program for cervical cancer should encompass all levels of the health system and requires appropriate coordination and collaboration among them, keeping in mind the ultimate goal, which is the woman's health.**
- **In order for the screening programme in Albania to have a high coverage, it should incorporate the elements of systematic information and personal invitation for target women. To reach the required high coverage there are needed the measures which aim at lowering of financial barriers (negligent costs for invited women) and geographical barriers (screening tests and gynaecological examinations spread geographically in the territory close to where women live, while keeping centralized the tests reading).**
- **The organized screening program should be applied gradually to encompass at the end all the regions of the country, while assuring sustainability through appropriate regulations and support from Ministry of Health.**

Based on the best scientific evidence on cervical cancer control and the most recent European guidelines for assuring quality on cervical cancer screening (European Union) and guidelines of basic practice for comprehensive control of cervical cancer (World Health Organisation), The Conference proposes that the measures to be undertaken in this field in Albania should be based on the following principles:

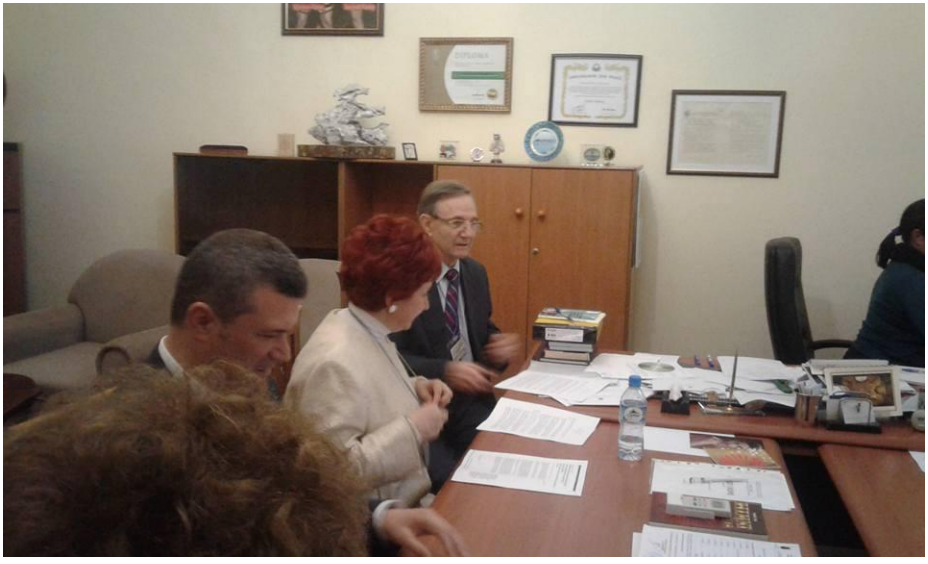
- **The level of development of the health system in our country allows moving towards the strategy 'screening – diagnoses – pre-cancer treatment' instead of 'screening-treatment' strategy. This way, the program would contribute to keep at a minimum the unnecessary invasive interventions;**
- **Primary Health Care Centres are the most appropriate venue for first contact of target women with health personnel and providing the services of counselling and taking of biological sample for screening test. General practitioners, midwifes and nurses working in these health centers, to carry out a large scale screening program, should be supported with training based on national guidelines/protocols;**
- **The gaps related to diagnoses and treatment components, at public health institutions should be minimized in order to avoid the risk of the program losing credibility in the eyes of public. More efforts should be**

undertaken to continuously improve the quality of services of diagnoses and treatment for cervical cancer in public health institutions of Albania;

- **To increase the efficiency and to control the quality, the primary screening tests reading should be centralized in Tirana;**
- **In addition to pap-test, it should be seriously taken into consideration the introduction of high risk HPV test as a primary screening test, based on its high effectiveness and practicality during large scale application of screening program;**
- **It is not cost effective the parallel application of two primary screening tests, but it is recommended that one of the tests to be used as triage, for all positive cases identified during the primary testing.**

Finally, participants in the conference, after being informed about the best experiences and practices nationally and internationally, agree about the need of inter-institutional organization and coordination in this field;

- **Ministry of Health should lead the process of setting up the national program for screening of cervical cancer, through appropriate regulation and dedication of recourses.**
- **University Hospitals, in collaboration with Institute of Public Health and National Centre for Health Quality and Safety, should prepare the standards, instruments of continuous quality improvement and indicators of monitoring and evaluation of the program. Collaboration with international experts in this field should be helpful. A board with representatives from the abovementioned institutions and approved by Minister of health would facilitate the follow up and sustainability of the process.**




INTERNATIONAL SCIENTIFIC CONFERENCE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AKADEMIA E SHKENCAVE E SHQIPËRISË
1972
TIRANË

CURRICULUM VITAE

Nazira ARTYKOVA

Dr. Nazira Artykova attended the State Medical University 1975 from 1981 and graduated as Medical Doctor.

She received her PhD in Obstetrics and Gynecology from 1984to 1986 from the Kharkov Medical University.

Dr. Nazira Artykova attended the Russian Academy of Medical Sciences, Professor of Obstetrics and Gynecology from1995-1996.

She is a health cluster coordinator to North Korea. She managed UNCT activity during natural disasters (Floods) and humanitarian assistance to North Korea through UNOCHA/CERF, SEARHEF since February 1997 and the WHO representative to Albania since June 2016.

She was Deputy Director Health Systems Consultant, CARE International from April 1995to September 1995.

She has over 100 scientific public health publications.

Manuela BELLO

Dr. Bello is the UNFPA Assistant Representative since 2003.

She graduated in General Medicine from the University of Tirana in 1989 and the Faculty of Social Sciences in the area of Gender and Development in 2008.

She has over 30 publications.

Arjola XHAJA

Dr.-medic

Dr. Arjola Xhaja was born in Tirana, Albania in 1972.

She studied Human Medicine from 1992 – 1999 in Tirana and Bucharest, Romania and graduated as Medical Doctor from the Carol Davila in 1999. She specialized in Obstetrics and Gynecology in 2004.

She was trained in the area of Obsterics / Gynecology in Erfurt, Friedrichroda, Parchim, also senior function in Hanau, Germany from 2004 to 2008.

She works a senior doctor in the area of Obstetrics / Gynecology, at MVZ Cytomol, since 2008. She is overqualified in the gynecological exfoliativ cytology. She is responsible for the quality of diagnoses and organization of several training events and Workshops in Germany, Switzerland and Rumania.

She has over 20 oral presentations and different speeches (Germany and International) 5 papers as author and co-author. She is part of several investigation teams for major scientific and clinical studies in Bucharest and by Cytomol on cytology and Immunocytochemistry stain and HPV testing.

Alban YLLI

MD, MSc, PhD, Assoc. Prof

Dr. Alban Ylli was born on 21 September 1968 in Tirana, Albania.

He graduated as general Practioner from the Faculty of Medicine, University of Tirana in 1993 and received his Master degree in Epidemiology of Health Services Faculty of Medicine, University, Tor Vergata, Rome, Italy (St Egidio Scholarship –one year) in 1995**.** Dr. Ylli specialized on non-infectious diseases research. "Bus Sante 2000 project", Department of community medicine, University of Geneva (Confederation Scholarship - one year) and in studies on prevention policies from the Institute of Health Sciences, Oxford University, (British, Chevening Scholarship – one year) in 2000. He is an Associate Professor since September 2014.

Dr. Ylli was the responsible for Chronic Diseases Unit at the Institute of Public Health, Tirana from July 1996 to September 1999. He is lecturer at the Public Health Department, Faculty of Medicine, Tirana, since September 1997.

He has been involved in several evaluation programs, research activities both nationally and internationally. In addition, he is member of several national and international organizations and editorial boards.

He is the author and coauthor of many study reports, public health manuals or books, oral presentations at different national and international conferences in English and Albanian.

Hans IKENBERG, M.D., Ph.D.

Dr. Hans Ikenberg was born in Würzburg, Germany in 1954.

He studied Studies of Human Medicine at Würzburg, Caen (France) and Munich from 1973 to 1980.

He was a research assistant at the Institute of Virology of the University of Freiburg and the German Cancer Research Centre, Heidelberg (H. zur Hausen), from 1981-1984. His work was focused on the detection and molecular cloning of human papillomaviruses in genital tumours.

He specialized in Gynaecology and Obstetrics from 1984-1992, from the Department of Gynecology and Obstetrics, University of Freiburg.

He was consultant / senior consultant and Head of the Tumour Laboratory in the Department of Gynaecology and Obstetrics of the University of Freiburg from 1992 to 2000. Work was focused on the pathogenesis, diagnosis and therapy of HPV-associated gynaecological tumours.

He was awarded the Schmidt-Matthiesen-Price of the Society for Gynecological Oncology (AGO) and the German Cancer Society (Deutsche Krebsgesellschaft) in 1994, received the University Research (Dr. med. habil. = Ph.D.) and Teaching Certification for Gynaecology and Obstetrics Privatdozent) in 1996 and Board Certification of "Special Surgical Gynaecology", "Special Obstetrics Perinatal Medicine" and "Gynaecologic Cytology" in 2000.

He is partner in two Medical Partnerships since 2000, Börsch – Breinl – Ikenberg, Frankfurt (named CytoMol since 1.4.2007) specialized in cytology and molecular diagnostics, since 2005.

He is the author and co-author of 66 papers in peer-reviewed-journals, 32 review papers and book articles, 104 oral presentations with citable abstracts, more than 400 educational speeches, lead investigator in major scientific and clinical studies on cytology and HPV testing, organizer of multiple conventions on HPV and member of several expert and guideline committees.

Klodian RJEPAJ

Ministry of Health

Dr.Klodian Rjepaj was born in Vlora, on March 16, 1971. He is married to Mrs. Gentiana Rjepaj and has two children, Egir and AnaGreis. He attended the Faculty of Medicine, Tirana, Albania as general practitioner and specialized in the area of public health. Received his Master Degree at the Faculty of Medicine, in the field of Public Health and the online Master Degree from the University of Manchester (UK). Mr. Rjepaj has currently finished his PhD in the area of Public Health. From 1998 to 1999 he worked as general practitioner in two villages of Vlora. In March 1999 he volunteered in Kukës and Has during Kosovo's crises.

From 1999 – 2001 he was the Chairman of the International Association of "International Medical Corps" (IMC). From 2002 to 2006 he was the National Coordinator of the Project on Mental Health run in the framework of the Pact of Stability.

In 2002 he was the Medical Chairman of the International Medical Corps (IMC) in Moscow, Russia. He was the leader of the National Program about HIV/AIDS/IST, Institute of Public Health and Coordinator of Global Fund against HIV HIV/AIDS in Albania.

In 2009-2012, he was Director of the Cabinet at the Ministry of Health and Chairman of the Coordination Committee for the fight against HIV / AIDS and Tuberculosis in Albania. He speaks English, Italian, French and Greek.

e-mail: klodian.rjepaj@shendetesia.gov.al

Lila SHUNDI

Assoc. Prof

Assoc. Prof. Lila Shundi was born on 5 January 1967 in Tirana, Albania.

She currently works at the Institute of Public Health, Tirana. She is currently the Head of the Laboratory of Molecular Biology, Department of the Control of Infective Diseases, Institute of Public Health, Tirana.

She attended the University of Tirana from 1985 to 1985 and graduated in Biology and Chemistry from the University of Tirana in 1989.

She studied Romanian at the Faculty of Foreign Languages, Polytechnic University of Bucharest from 1993 to 1994.

She received her PhD in genetics from the Faculty of Biology, University of Bucharest, Romania in 1999.

She received her Post-doctorate degree in the area of Molecular Epidemiology from the Laboratory of Molecular Epidemiology, Institute Cantacuzino, Bucharest, Romania in 1999.

She is a scientific researcher at the Department of the Control of Infective Diseases, Institute of Public Health since 2002.

Prof. Shundi was the Head of the Laboratory Sector Department of the Control of Infective Diseases, Institute of Public Health from 2006 to 2012.

Scientific researcher at the Center of Molecular Diagnostics and Genetic Diseases, University Hospital of Obstetrics-Gynecology, Queen Geraldinë, Tirana from 2004 to 2006 Albania, scientific researcher at the Department of Microbiology and Physiology, Institute for Biologic Research, Albanian Academy e of Sciences, Tirana from 2000 to 2002, Social worker and translator for the International Catholic Migration Commission (ICMC) in Albania, from 1999 to 2000, Department of Genetics, Faculty of Biology, University of Bucharest from 1994 to 1998, Laboratory of Molecular

Epidemiology, Institute Cantacuzino, Bucharest, Romania in 1999 and, Teacher of biology and chemistry at the "Ismail Qemali," High School, from 1990 to 1993.

Prof. Shundi's academic activity relates to teaching of Molecular diagnostics of infective diseases, Medicine and Pharmaceutical Biotechnology Molecular Diagnostics and Laboratory Microbiology and Practices of Microbiology Laboratory at the Catholic University, Our Lady of Good Counsel, Tirana, Albania, University of Tirana and American University of Tirana, Albania, respectively.

Prof. Lila Shundi is the author and coauthor of over 40 papers in English, Albanian, Romanian etc.

Majlinda IKONOMI
Assoc. Prof.

Prof. Majlinda Ikonomi was born on 7 May 1994 in Berat, Albania.

She is currently, Associate Prof. of Pathology at the Faculty of Faculty of Medicine, University of Medicine, Tirana, Albania

She studied general medicine at the Faculty of Medicine University of Tirana, from 1993 to 1999.

She received her Master in Anatomic Pathology in 2002 and her PhD in Medical Sciences with the thesis "Chronic gastritis, H. Pylori and its role" in 2008.

She is lecturer at the department of Anatomic Pathology, University of Tirana, Faculty of Medicine.

She worked as specialist of pathologic anatomy at University Hospital Centre, "Mother Theresa" from 1999 to 2010.

Chief of the Laboratory of Anatomic Pathology, Oncology Service, UHC "Mother Theresa", since 2010 and Head of Pathology Laboratory, Hygeia Hospital Tirana, Albania since 2012. She attended a training course on Osteo-medullary biopsy, Cytopathology from June to September, 2001 at the University of Bari, Italy, course on Cytopathology at the Hospital Saint-Antoine, Paris France from October to December 2002, course on Pap-smears in 2007 at the University of Bari, Italy, course on Cancer Prevention at the National Cancer Institute, Bethesda, USA from June to August 2007, course on Pathologic Anatomy, in July 2011 at the Centro di riferimento Oncologico Aviano, Italy.

She is the author and co-author of many papers.

Mirela RISTA (MINO)

Assoc Prof.

Mirela Rista (Mino) was born on 17 August 1966 in Tirana, Albania.

She studied general medicine at the Faculty of Medicine, University of Tirana, Albania. She carried out her Post University studies at the University Hospital of Obstetrics-Gynecology, Tirana, Albania in 1993. She carried out her Post-university advanced studies on obstetrics-gynecology and on ekographic examination at Hospital University Saint-Antoine, Paris, France from January-October 1994, on Colposcopy at the Department of Obstetrics-Gynecology Sciences, University of Studies, Modena, Italy from 20 December 1996 to 30 January 1997, on "reproductive Health and Family Planning" from 11 October to 12 November1999, in Debrecen, Hungary, on histeroscopic examination and Fluximetry in Echography at Faculty of Medicine, University of Studies, Aquila, Italy, from 20 June to 3 August 2005 and on "Mother and Child Health Care in Shanghai, China from 28 May-11 June 2007.

She has been lecturer of Obstetrics – Ginecology, Department of Family Doctor Faculty of Medicine,Tirana, Albania since March 2015 and External Lecturer from August 2012 – March 2015.

She is the author and coauthor of over 45 scientific papers in English, Albanian and Romanian.

Đurđica OSTOJIĆ, MD, PhD

Dr. Đurđica OSTOJIĆ attended the School of Medicine, University of Rijeka, Yugoslavia October 1973 to October 1978.

She specialiazed in Pediatrics at the Children's hospital Kantrida Rijeka, Yugoslavia from 1983 to1987 and carried out her master studies from 1986 to 1988 at the Clinical Pathophysiology - immunology department School of Medicine, University of Rijeka, Yugoslavia. Her master thesis was entitled "Pollinosis and hyposensibilisation in children", the PhD at the Faculty of Medicine, University of Belgrade, Serbia, in 2006, with the PhD thesis entitled "Determination of markers of inflammation in asthma in children". The same year she was awarded *'Primarius'* from the Ministry of Health of the Republic of Montenegro.

She works at the Public Health Institute of Montenegro, Center for Control and Prevention of non-communicable diseases since November, 2013. She worked for the Health Insurance Fund of Montenegro from November, 2007 to November, 2013, Private Clinic "Sanicard" Podgorica, Montenegro as doctor pediatrician from April, 2007 to October, 2007, Health Centre Podgorica, Montenegro as doctor pediatrician from July , 1980 to

April , 2007 and, Clinical Centar 'Dr Zdravko Kučić' Rijeka, Yugoslavia from May, 1979 to May 1980 .

She is the author of many scientific papers.

ELENA KJOSEVSKA
PhD, MD

Prof. Dr. Elena Kosevska, 25.06.1958, was born in Skopje. She graduated from the Medical faculty Skopje 1984, 1993 becomes a specialist in social medicine with the organization of health system, and 2004 defended a doctoral dissertation entitled: The role of health education in prevention of risk factors for ischemic heart disease.

Professional education: CDC-Atlanta, USA: International Management of Public Health, School of Public Health-Tver, Russia, School of Public Health *Jerusalem,* Israel, Edinburg, London, Copenhagen, Tirana, Belgrade, Zagreb, Helsinki, Luxemburg, Porto, Sofia, Budapest, Bilthofen, Salzburg, etc.

Her domestic and international nominations and functions have been the National health promotion counterpart with WHO, until 2015, National counterpart for the WHO survey on prevalence of tobacco use among youth in Macedonia (GYTS Global Youth Tobacco Survey), Expert in charge of cooperation with MOH, EUROSTAT, WHO, UN, State Statistical Office for submission of health statistical data, health indicators (HFA-DB), Millennium goals, Member of the Technical Group on morbidity in EUROSTAT, Member of the Working Group on Statistics-NPAA, Government of Republic of Macedonian, Chairman of the Association of Specialist Doctors in Social Medicine at the Macedonian Medical Association, 2006-2014, Member of EUPHA, WFPHA, BENA, etc. and, Technical focal point for tobacco control and counterpart with WHO, 2015.

Professional experience are related to planning, monitoring and evaluation of health promotion and education in the Republic of Macedonia, evaluation of health status and health care of the population, Roma health, Maternal and Child Health, Youth Health, development and implementation of national strategies and law documents: Health Strategy in the Republic of Macedonia by 2020, Strategy and action plan for tobacco control, Strategy for prevention and control of non communicable diseases, Strategy to reduce peri-natal mortality and ensuring safe motherhood, Strategy for sexual and reproductive health in the Republic of Macedonia-coordinator, Law for health care, Law for public health, Law for medical evidence in the health sector etc. and, development and implementation of national promotional and preventive programs (Program for preventive health care, Program and Campaign "Health for All", Program to reduce the malignant neoplasms of the reproductive organs in women and Campaign "Outwit the cancer, be a healthy

woman," Program and action plan for control of breast cancer, etc.), development of management criteria for evaluation the manager's knowledge and skills, etc.

Educational activity concern teaching at the Department for Social Medicine, Medical Faculty Skopje, lecturing at the Medical Faculty of Skopje - The Department of general medicine, responsible for teaching the subject of Health promotion and health education of three-year study of nurses, radiology technologists, physiotherapists and speech therapists, Medical Faculty Skopje, teaching in Social medicine at the Faculty of Dentistry Skopje, teaching at the School of Public Health for the subject Health Promotion, coordinating the evaluation studies ECTS, I yr. studies in general medicine, Medical Faculty Skopje, 2007 and mentoring PhD students of medicine and public health, master's in public health and specialist's studies in Social medicine, mentor of Roma students at the Medical Faculty Skopje

Research activities relate to Project Manager / Coordinator: (GYTS-2002, 2008, 2016), Healthy schools, Together for young, Medical map, Patient satisfaction-2006 - Associate of the main researchers in the projects: Implementation of ICD-10, reforms in the health sector, EPI-Program, Rehabilitation of patronage services, Reproductive health, A strategic assessment of conditions in abortion and contraception, Action plan for the decade of Roma population -section of health promotion, Assessment of needs for the opening of youth friendly services: (Youth Friendly Services), Healthy Municipalities, Health Behavior in School - Age Children (HBSC) in 2010 and 2014, Project for assessment of reproductive health and implementation of woman rights in the Republic of Macedonia, project: "Reorienting strategies, programmes and activities on MDG 4 and 5 towards greater health equity with an explicit but not exclusive focus on the Roma population", project: "Supporting and Scaling up Roma health mediators programme in Macedonia", project "Programme for medical scholarship for Roma students" at the universities supported by Open Society Foundations, etc.- Author / co-author of 108 professional and scientific papers in the field of public health - Supervisor of 7 master's, 3 PhD 1 specialist' works in the field of public health and medical science- Reviewer of professional and scientific papers published in the Journal of the Macedonian medical association-Macedonian medical review, Physioacta journal published by the Association of physiologists of RM, Medicus, journal published by the Association of Albanian Doctors in Macedonia and Contributions by Macedonian Academy of Sciences and Arts (MASA), Archives of Public Health from the Institute for Public Health of the Republic of Macedonia, Social Medicine, journal published by Macedonian Association for Social Medicine.

Awards and honours:

Acknowledgment - National Institute for Health Protection in Skopje

Gratitude, Diploma, Plaque, "Povelba Dr. Trifun Panovski" (2015) - Macedonian Medical Association

MARTIN KRESTANPOL

Dr. Martin Krestanpol PhD. graduated from Masaryk's University in Brno, Czech Republic (Molecular Biology and Genetics), 1986. Dr. Martin Krestanpol's scientific work was dedicated to molecular detection of atypical mycobacteria (1986-1994; National Institute of Public Health Prague, Royal Institute for Tropical Medicine Amsterdam). Dr. Martin Krestanpol has been working in the field of molecular diagnostics from 1994. The main focus was dedicated to HPV detection and the cervical cancer prevention since 1996, including the coordination of large HPV trials across Europe within Digene (Finnish trial, Pekka Nieminen, Ronco NTCC Italian Italy, ARTISTIC UK Trial, POBASCAM (The Netherlands Trial etc.). Martin Krestanpol works currently as a Freelance Consultant.